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[REVIEW]

Distribution and Function of Gonadotropin-Releasing Hormone (GnRH) in the Teleost Brain

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INTRODUCTION

Gonadotropin-releasing hormone (GnRH) is a decapeptide originally isolated from pig and sheep hypothalami as a physiologic regulator of luteinizing hormone (LH) release from the pituitary (Matsuo *et al.*, 1971; Burgas *et al.*, 1972). At present, nine GnRH molecules have been characterized and named for the vertebrate species in which they were first identified (Fig.1): mammalian GnRH, chicken GnRH-I (King and Millar, 1982a,b; Miyamoto *et al.*, 1982, 1983), chicken GnRH-II (Miyamoto *et al.*, 1984), salmon GnRH (Sherwood *et al.*, 1983), lamprey GnRH-I (Sherwood *et al.*, 1986), lamprey GnRH-III (Sower *et al.*, 1993), catfish GnRH (Bogerd *et al.*, 1992; Ngamvongchon *et al.*, 1992), dogfish GnRH (Lovejoy *et al.*, 1992), and seabream GnRH (Powell *et al.*, 1994). They have been identified in vertebrate brains and constitute a family of structurally-related decapeptides. During the 500 million years of vertebrate evolution, the primary structure of GnRH has been remarkably conserved.

It is generally accepted that GnRH regulates synthesis and release of pituitary gonadotropin (GTH) (see King and Millar, 1992; Sherwood *et al.*, 1993). In addition, GnRH can act as a neuromodulator, and administration of exogenous GnRH facilitates sexual behavior in many species (see Pfaff *et al.*, 1987).

In mammalian, avian, reptilian, and amphibian animals, GnRH is conveyed to the pituitary via the hypothalamo-hypophyseal portal vessels. In mammals, pulsatile release of mammalian GnRH (mGnRH) by hypothalamic neurons stimulates GTH secretion from the pituitary. Teleost fishes lack the median eminence. Instead GnRH neurons are found to directly innervate the pituitary. It is therefore interesting to examine GnRH systems in teleost fish in view of comparative endocrinology.

Immunocytochemistry and radioimmunoassay (RIA) have been utilized for studying the distribution and function of GnRHs in the brain. In addition, recent progress in molecular biological methods has enabled us to study expression of GnRH genes using hybridization techniques (see Urano and Hyodo, 1990).

This review will focus on the roles of the GnRHs in the teleost brain, particularly in salmonid fishes.

IDENTIFICATION OF GnRH

In teleost fish, the primary structure of salmon GnRH (sGnRH) was first determined from chum salmon, *Oncorhynchus keta* (Sherwood *et al.*, 1983), followed by subsequent determination of catfish GnRH (Ngamvongchon *et al.*, 1992) and seabream GnRH (Powell *et al.*, 1994).

The presence of two types of GnRH, sGnRH and chicken GnRH-II (cGnRH-II), in the teleost brain was first demonstrated in the goldfish, *Carassius auratus*, by employing high performance liquid chromatography (HPLC) in conjunction with RIA (Yu *et al.*, 1988). Recent studies have shown that in teleosts, more than one type of GnRH molecule exist even within the same species, and all of the teleosts examined thus far have cGnRH-II, whereas the second form is either sGnRH, mGnRH, catfish GnRH, or seabream GnRH. Existence of three forms of GnRH, i.e. sGnRH, cGnRH-II, and seabream GnRH, was recently reported in the brain of the seabream, *Sparus aurata* (Powell *et al.*, 1994) and African cichlid, *Haplochromis burtoni* (White *et al.*, 1995). The existence of multiple forms of GnRH in the brain suggests that each GnRH has a different distribution and function.

Differential distribution of multiple forms of GnRH in discrete brain areas was examined by RIA in several fishes in order to clarify their functions in the brain. Okuzawa *et al.* (1990) first measured sGnRH and cGnRH-II contents in the discrete brain regions of the rainbow trout, *Oncorhynchus mykiss*, using specific RIAs. These authors found that the contents of both types of GnRHs varied in different brain regions. The levels of sGnRH were higher than those of

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	1 2 3 4 5 6 7 8 9 10
Mammalian	pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2
Chicken-I	pGlu-His-Trp-Ser-Tyr-Gly-Leu-Gln-Pro-Gly-NH_2
Chicken-II	pGlu-His-Trp-Ser-His-Gly-Trp-Tyr-Pro-Gly-NH_2
Salmon	pGlu-His-Trp-Ser-Tyr-Gly-Trp-Leu-Pro-Gly-NH_2
Catfish	pGlu-His-Trp-Ser-His-Gly-Leu-Gln-Pro-Gly-NH_2
Seabream	pGlu-His-Trp-Ser-Tyr-Gly-Leu-Ser-Pro-Gly-NH_2
Dogfish	pGlu-His-Trp-Ser-His-Gly-Trp-Leu-Pro-Gly-NH_2
Lamprey-I	pGlu-His-Tyr-Ser-Leu-Glu-Trp-Lys-Pro-Gly-NH_2
Lamprey-III	pGlu-His-Trp-Ser-His-Asp-Trp-Lys-Pro-Gly-NH_2

Fig. 1. Amino acid sequences of the identified GnRH peptides in vertebrates.

cGnRH-II in the olfactory bulbs, the telencephalon, the hypothalamus, the optic tectum-thalamus and the pituitary, whereas the cerebellum and the medulla oblongata contained much more cGnRH-II than sGnRH. Especially of note, cGnRH-II was undetectable in the pituitary. This is also true for masu salmon, *Oncorhynchus masou* (Amano *et al.*, 1992, 1993). These results suggest that, of the two GnRHs, only sGnRH is involved in GTH secretion. As an example, sGnRH contents and cGnRH-II contents in each region of the brain of ovulated female masu salmon are shown in Fig. 2.

In the goldfish, sGnRH was distributed in a larger amount in the olfactory bulbs, the telencephalon, the hypothalamus, and the pituitary than in the other regions, whereas cGnRH-II was distributed widely throughout the brain with highest concentrations in the medulla oblongata. The major difference between salmonid fishes and goldfish is that goldfish pituitary contains cGnRH-II (Kobayashi *et al.*, 1992, 1994).

In the European eel, *Anguilla anguilla*, mGnRH levels were higher than cGnRH-II levels in the pituitary, the olfactory lobes together with the telencephalon, and the diencephalon together with the mesencephalon, while the opposite results were obtained for the posterior part of the brain. Of interest, cGnRH-II levels in the pituitary were slightly above the detectable limit (Dufour *et al.*, 1993).

These studies demonstrated that sGnRH (or mGnRH) and cGnRH-II are differently distributed in the brain, and also necessitated to investigate the localization of GnRH neurons and the changes in GnRH levels during gonadal maturation.

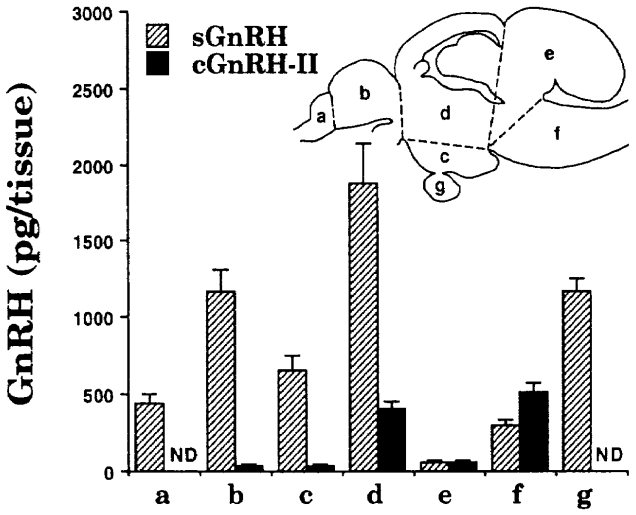

Fig. 2. Contents of sGnRH and cGnRH-II per brain region in ovulated female masu salmon. Schematic diagram of a sagittal section of masu salmon brain shows dissection for the determination of GnRH. Letters represent the following brain areas: a, olfactory bulbs; b, telencephalon including the POA; c, medio-basal hypothalamus; d, optic tectum-thalamus and dorsal hypothalamus; e, cerebellum; f, medulla oblongata; g, pituitary.

IMMUNOCYTOCHEMICAL DISTRIBUTION OF GnRH NEURONS

There have been many immunocytochemical studies relating to the GnRH system in teleost brains. Serious problems here are that most of these studies used antiserum against mGnRH, for example, in the rainbow trout (Goos and Murathanoglu, 1977; Schäfer *et al.*, 1989), in the goldfish (Stell *et al.*, 1984; Kah *et al.*, 1984), in the platyfish, *Xiphophorus maculatus* (Münz *et al.*, 1981; Halpern-Sebold and Schreibman, 1983), in the eel (Nozaki *et al.*, 1985; Grober *et al.*, 1987), in the catfish, *Clarias batrachus* (Subheader and Krishna, 1988), in the labrid fish (Grober and Bass, 1991). Some researchers used antiserum against sGnRH, without any characterization of cross reactivity to cGnRH-II, i.e., in the goldfish (Kah *et al.*, 1986), in the green molly, *Poecilia latipinna* (Batten *et al.*, 1990), and in the rainbow trout (Bailhache *et al.*, 1994). Since, as is mentioned above, more than one type of GnRH molecule exists even within the same species, several authors have immunocytochemically examined the distribution of multiple GnRH forms in the teleost fish brains (Amano *et al.*, 1991; Montero *et al.*, 1994; Yamamoto *et al.*, 1995; Kim *et al.*, 1995).

The distribution of sGnRH immunoreactive (ir) neuronal somata and fibers, and that of cGnRH-II-ir neuronal somata

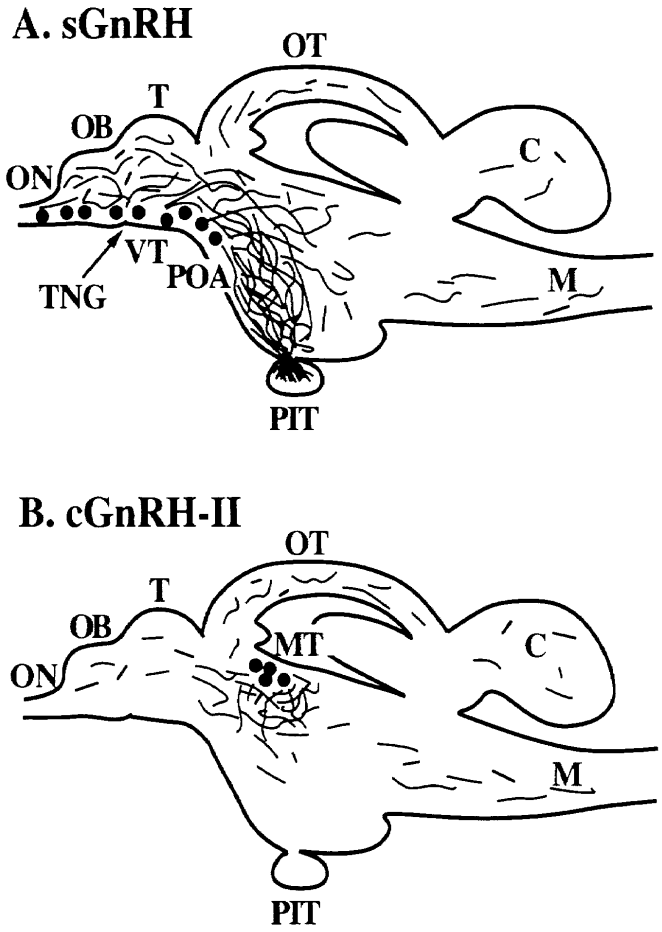

Fig. 3. Schematic illustration of the distribution of (**A**) sGnRH-ir neuronal somata (filled circles) and fibers, and (**B**) cGnRH-II-ir neuronal somata (filled circles) and fibers in masu salmon. ON, olfactory nerve; OB, olfactory bulb; TNG, terminal nerve ganglion; T, telencephalon; VT, ventral telencephalon; POA, preoptic area; OT, optic tectum-thalamus; PIT, pituitary; MT, midbrain tegmentum; C, cerebellum; M, medulla oblongata.

and fibers in the masu salmon, are summarized in Fig. 3 (Amano *et al.*, 1991). sGnRH-ir neuronal somata were scattered in the olfactory nerve (ON), the olfactory bulb (OB), between the olfactory bulb and the part of telencephalon which corresponds to the terminal nerve ganglion (TNG), the ventral telencephalon (VT), and the preoptic area (POA). sGnRH-ir fibers were distributed in various brain regions from the OB to the spinal cord. sGnRH-ir fibers directly innervated the pituitary. cGnRH-II-ir neuronal somata were found in the midbrain tegmentum located rostral to the motoneurons of the oculomotor nerve. The distribution of cGnRH-II-ir fibers was basically similar to that of sGnRH-ir fibers except for the absence of cGnRH-II-ir fibers in the pituitary. The number of cGnRH-II-ir fibers in the brain were much fewer than those of sGnRH. The distribution of sGnRH-ir neuronal somata in chum salmon was consistent with that in masu salmon (Kudo *et al.*, 1996). These results suggest that, in salmonid, sGnRH not only regulates GTH secretion but also functions as a neuromodulator, whereas cGnRH-II functions only as a neuromodulator. Nonetheless, both GnRHs can stimulate release of GTH I and GTH II from the pituitary *in vitro* in sockeye salmon, *Oncorhynchus nerka* (Amano, 1993).

Differential localization of two types of GnRH, mGnRH and cGnRH-II, were reported in the European silver eel (Montero *et al.*, 1994). mGnRH-ir neuronal somata were observed in the OB, the nucleus olfactoretinalis, the VT, the POA, and the mediobasal hypothalamus. mGnRH-ir fibers were distributed in many parts of the brain and also in the pituitary. cGnRH-II-ir neuronal somata were detected in the midbrain tegmentum, and very few cGnRH-II-ir fibers were observed in the pituitary.

In the goldfish, sGnRH-ir neuronal somata were localized in the TNG area between the ON and the OB, the VT, the POA, and the hypothalamus. cGnRH-II-ir neuronal somata were observed in the TNG, the VT, the POA, the hypothalamus and the midbrain tegmentum. Both sGnRH-ir and cGnRH-II-ir fibers were distributed not only in the hypothalamus and the pituitary but also in various brain areas from the OB to the spinal cord (Kim *et al.*, 1995). These results suggest that both sGnRH and cGnRH-II not only regulates GTH secretion but also function as neuromodulators. Indeed, both forms of GnRH have been shown to induce *in vitro* release of GTH II and growth hormone (GH) in goldfish (Marchant and Peter, 1989; Marchant *et al.*, 1989; Chang *et al.*, 1990; Habibi *et al.*, 1992). Although both GnRHs bind to the same pituitary receptors (Habibi, 1991; Habibi *et al.*, 1992), they appear to induce hormone release via somewhat dissimilar mechanisms (Chang *et al.*, 1991, 1992). Moreover, stimulation of sGnRH and cGnRH-II on GTH II synthesis was shown in goldfish (Khakoo *et al.*, 1994); sGnRH, but not cGnRH-II, increased the accumulation of GTH IIβ and GTH α mRNA in sexually regressed fish, whereas both sGnRH and cGnRH-II stimulated accumulation of GTH IIβ and GTH α mRNA in sexually mature fish.

An approach requisite for clarification of the function of each GnRH-ir neuronal group is to examine its projection area in the brain. Recently, three different projection patterns were demonstrated in dwarf gourami, *Colisa laria*. GnRH neurons in the TNG which showed strong sGnRH and weaker cGnRH-II immunoreactivity projected to wide areas of the central nervous system from the OB to the spinal cord; sGnRH neurons in the POA projected only to the pituitary, and cGnRH-II neurons in the midbrain tegmentum projected mainly to brain regions posterior to the hypothalamus and the spinal cord (Yamamoto *et al.*, 1995). These different projection patterns indicate functional differentiation of GnRH neuronal groups.

CHANGES IN GnRH CONTENTS DURING GONADAL MATURATION

If GnRH is involved in gonadal maturation, GnRH contents would be expected to change during gonadal maturation. Several studies were conducted to measure brain GnRH contents by RIA in relation to gonadal maturation in eel (Dufour *et al.*, 1982), platyfish (Schreibman *et al.*, 1983), brown trout, *Salmo trutta* (Breton *et al.*, 1986), caribe colorado, *Pygocentrus notatus* (Gentile *et al.*, 1986), goldfish (Yu *et al.*, 1987), and

chinook salmon, *Oncorhynchus tschaawytsca* (Lewis *et al.*, 1992). However, the results were discordant. A clear correlation between brain GnRH contents and gonadal maturity was observed only in caribe colorado (Gentile *et al.*, 1986). Such discrepancies may be, in part, due to the use of RIAs employing nonspecific antibodies.

We investigated changes in sGnRH and cGnRH-II contents in the brain and pituitary of masu salmon from hatching through gonadal maturation for three years. During gonadal maturation, sGnRH levels in the telencephalon including the POA and the pituitary correlatively increased with the elevation in GTH II levels in the pituitary and the plasma (Fig. 4). cGnRH-II were undetectable in the pituitary. Further, no significant changes in the concentration were found in discrete brain areas during vitellogenesis and ovulation (Amano *et al.*, 1992). These results together with those of immunocytochemical studies suggest that sGnRH neurons in the telencephalon-POA are involved in gonadal maturation in masu salmon.

Recently, it was demonstrated that GnRH fibers originating from the TNG is not involved in GTH secretion and further in gonadal maturation in goldfish. The sGnRH contents in the brain except the OB markedly decreased by olfactory tract sectioning (OTX), whereas the cGnRH-II contents in the brain showed no clear changes. Despite large decreases in the brain sGnRH contents, gonadal maturation was not inhibited (Kobayashi *et al.*, 1992, 1994). In dwarf gourami, GnRH neurons in the TNG, possibly sGnRH neurons, may be the most extensively projecting GnRH neurons in the brain except in the pituitary (Oka and Matsushima, 1993). These results indicate that most of the sGnRH fibers in the brain originates from the TNG, and that sGnRH neurons in the TNG do not project to the pituitary. Thus, it is possible that changes in the sGnRH contents measured by RIA reflect the activities of GnRH neurons in the TNG. It should be noted again that the levels of GnRH measured by RIA must be considered as a summation of synthesis, release and degradation of GnRH at any point in development. Therefore, it is necessary to examine the expression of GnRH gene in order to clarify function of GnRH in reproduction.

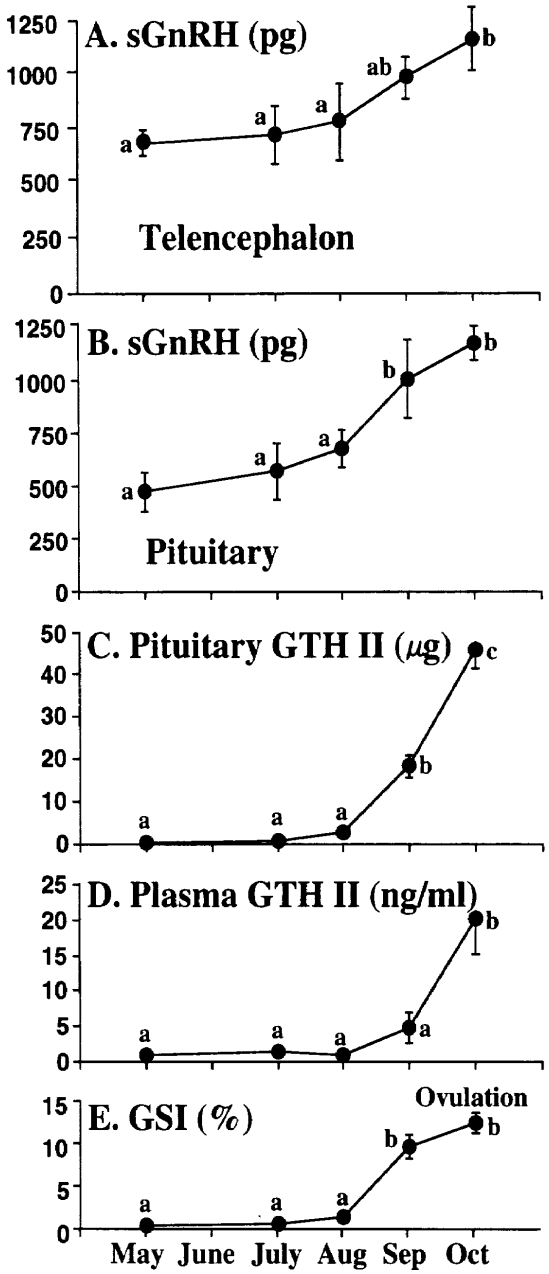

Fig. 4. Changes in (**A**) sGnRH contents in the telencephalon, (**B**) sGnRH contents in the pituitary, (**C**) GTH II contents in the pituitary, (**D**) GTH II concentrations in the plasma, and (**E**) gonadosomatic index (GSI) of 2-year-old female masu salmon. Means with differing letters differ significantly ($p<0.05$) in each panel.

MOLECULAR CLONING OF GnRH GENES

There are several methods for the examination of hormonal gene expression. Among them, *in situ* hybridization (ISH) can be used to analyze mRNA expression of tissues of interest directly on histological sections (see Urano and Hyodo, 1990). Since sGnRH neuronal somata are widely scattered in the brain, ISH in combination with immunocytochemistry is a strong tool to yield information on the dynamic aspects of the synthesis of GnRH in a certain neuron or region. However, the ISH method require a hybridization probe whose nucleotide sequence is complementary to the mRNA to be examined.

The cDNA coding for mGnRH was initially isolated from human placenta (Seeburg and Adelman, 1984), and subsequently, mGnRH genes have been cloned from several species (Adelman *et al.*, 1986; Mason *et al.*, 1986). Chicken GnRH-I (cGnRH-I) gene has been cloned from chicken (Dunn *et al.*, 1993). The cDNA for cGnRH-II has been isolated from teleost fish (White *et al.*, 1994; Bogerd *et al.*, 1994; Lin and Peter, 1996), the cDNA for sGnRH from several teleost fish (Bond *et al.*, 1991; Suzuki *et al.*, 1992; Klungland *et al.*, 1992; Okuzawa *et al.*, 1994; Grober *et al.*, 1995; Ashihara *et al.*, 1995; Suetake *et al.*, 1995; Lin and Peter, 1996), cDNA for catfish GnRH from African catfish, *Clarias gariepinus* (Bogerd *et al.*, 1994), and cDNA for seabream GnRH from African cichlid, *Haplochromis burtoni* (White *et al.*, 1995) and from red seabream (Okuzawa *et al.*, 1996).

In general, a GnRH precursor is composed of a signal peptide (SP), GnRH and a GnRH-associated peptide (GAP) which is connected to GnRH by a Gly-Lys-Arg sequence. The number of amino acid residues in SP and GAP in teleost GnRH precursors are shown in Table 1. The Gly-Lys-Arg sequence is considered to serve as a signal for proteolytic processing and C-terminal amidation (Nikolics *et al.*, 1988). It was reported that GAP showed GTH-releasing activity and prolactin-inhibiting activity in rat (Nikolics *et al.*, 1985), although prolactin-inhibiting activity of GAP is controversial. There have been no reports on the physiological functions of GAP in teleost fishes.

The distribution of GnRH ISH positive neurons has been examined in several teleost fishes (Suzuki *et al.*, 1992; Bailhache *et al.*, 1994; White *et al.*, 1995; Bogerd *et al.*, 1994). The distribution of sGnRH ISH positive neurons is consistent with that of sGnRH-ir neuronal somata in masu salmon (Suzuki *et al.*, 1992), rainbow trout and Atlantic salmon (Bailhache *et al.*, 1994). In African catfish, catfish GnRH ISH positive neurons were scattered in the ON, along both sides of the midline of the telencephalon, the POA, and in the infundibular stalk close to the pituitary, whereas cGnRH-II ISH positive neurons were observed in the midbrain tegmentum (Bogerd *et al.*, 1994). In African cichlid, sGnRH ISH positive neurons were detected in the TNG, whereas seabream GnRH ISH positive neurons were detected in the POA, and cGnRH-II ISH positive neurons were detected in the mesencephalon (White *et al.*, 1995). In red seabream, sGnRH ISH positive neurons were detected in the TNG, and seabream GnRH ISH neurons were detected in the POA (Okuzawa *et al.*, 1996). All these studies support the data obtained from immunocytochemistry.

REGULATION OF sGnRH mRNA EXPRESSION IN SALMONID FISHES

The cDNA sequences for sGnRH, which is involved in gonadal maturation through GTH secretion, have been isolated from several salmonid fishes (masu salmon, Suzuki *et al.*, 1992; Atlantic salmon, Klungland *et al.*, 1992; sockeye salmon, Ashihara *et al.*, 1995), making it possible to examine sGnRH synthetic activity using ISH. Hence, we examined the changes of sGnRH mRNA expression in context of several aspects of reproduction in salmonid fish.

(a) Changes in sGnRH mRNA expression during gonadal maturation

The widespread distribution of sGnRH-ir neuronal somata in the ventral part of the brain suggests that sGnRH has several functions in the brain in addition to the stimulation of GTH secretion (Amano *et al.*, 1991); however, it still remains obscure which sGnRH neurons are involved in gonadal maturation via GTH secretion. We therefore examined the changes of sGnRH mRNA expression during ovulation in 2-year-old female masu salmon (Amano *et al.*, 1995a). In this study, sGnRH synthetic activity were estimated by the number of neurons expressing sGnRH mRNA, the number of silver grains per neuron, and the total number of silver grains per unit area. The activity was increased in the VT and the POA during maturation in accordance with increases in GSI and plasma GTH II concentrations (Fig. 5). On the contrary, no significant changes were observed in the OB and the TNG. These results indicate that sGnRH neurons in the VT and the POA, but not in the OB and the TNG, are involved in the regulation of gonadal maturation possibly through GTH secretion. These results are consistent with the previous data that sGnRH concentrations in the telencephalon including the POA increased with gonadal maturation (Amano *et al.*, 1992, 1993).

In the dwarf gourami, GnRH originating from the TNG does not affect GTH secretion but rather has a function as a neuromodulator in the brain (Oka and Ichikawa, 1990; Oka, 1992; Yamamoto *et al.*, 1995). sGnRH originating from the TNG is also not considered to be essential for gonadal development in goldfish (Kobayashi *et al.*, 1992, 1994). It is

Table 1. Amino acid numbers of signal peptide (SP) and GnRH associated peptide (GAP) of GnRH precursors identified in teleost fishes

GnRH form	Species	SP	GAP	References
sGnRH	cichlid	23	54	Bond *et al.* (1991)
	masu salmon	23	46	Suzuki *et al.* (1992)
	Atlantic salmon	23	46	Klungland *et al.* (1992)
	red seabream	23	54	Okuzawa *et al.* (1994)
	sockeye salmon	23	46	Ashihara *et al.* (1995)
	plainfin midshipman	23	53	Grober *et al.* (1995)
	goldfish	23	58	Suetake *et al.* (1995)
	goldfish	23	58	Lin and Peter (1996)
cGnRH-II	cichlid	23	49	White *et al.* (1994)
	catfish	24	49	Bogerd *et al.* (1994)
	goldfish	24	49	Lin and Peter (1996)
catfish GnRH	catfish	21	46	Bogerd *et al.* (1994)
seabream GnRH	cichlid	22	63	Bogerd *et al.* (1995)
	red seabream	23	59	Okuzawa *et al.* (1996)

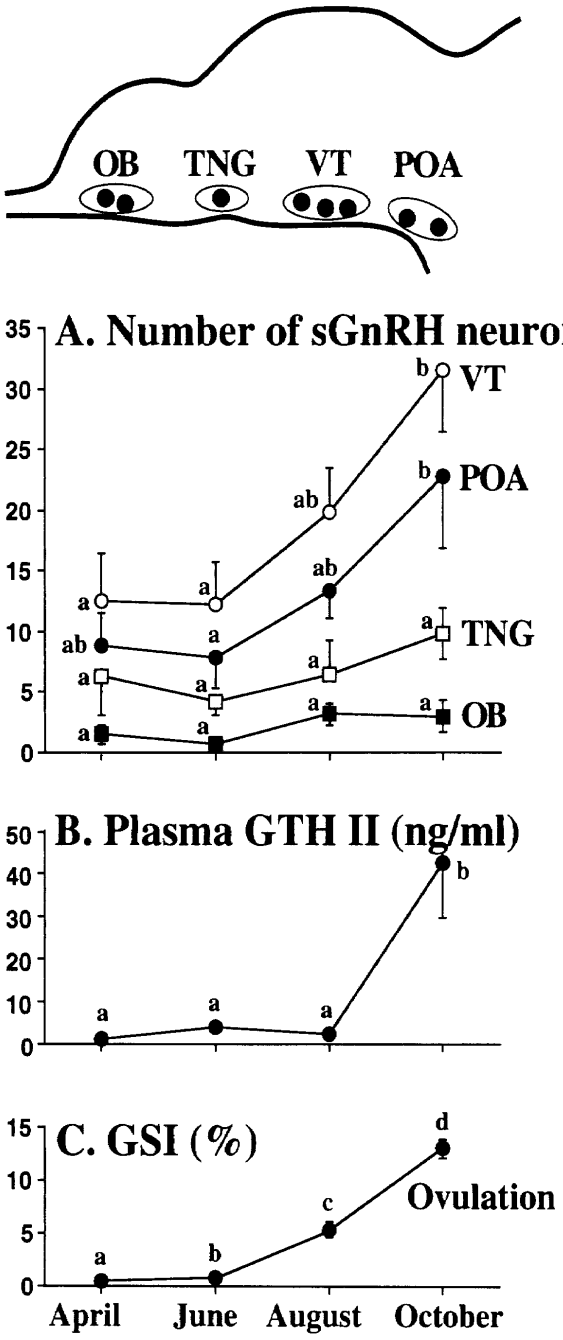

Fig. 5. Changes in (**A**) the number of neurons expressing sGnRH mRNA in the brain (OB, TNG, VT, POA), (**B**) plasma GTH II concentrations, and (**C**) GSI of 2-year-old female masu salmon. Means with differing letters differ significantly ($p<0.05$) in each panel.

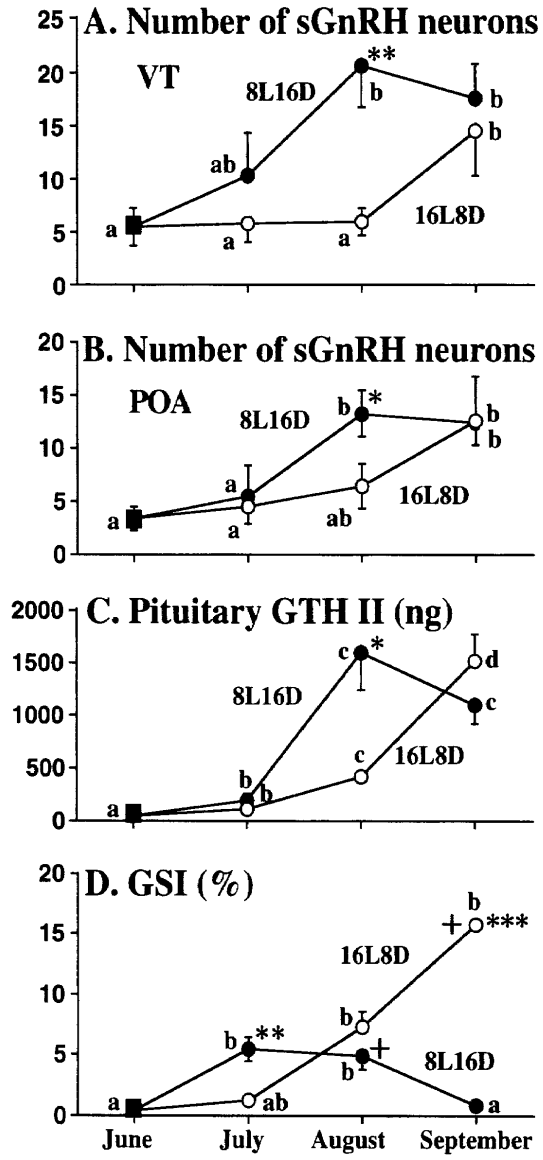

Fig. 6. Changes in (**A**) the number of neurons expressing sGnRH mRNA in the VT, (**B**) the number of neurons expressing sGnRH mRNA in the POA, (**C**) pituitary GTH II contents, and (**D**) GSI of underyearling male masu salmon reared under 8L16D or 16L8D. $^{*}p<0.05$; $^{**}p<0.01$; between the 8L16D and 16L8D group in each month. Means with differing letters differ significantly ($p<0.05$) in each panel.

also possible that sGnRH neurons in the TNG function to only modulate neuronal activity in masu salmon.

(b) Changes in sGnRH mRNA expression induced by photoperiod manipulation

Gonadal maturation in salmonid fish occurs in autumn, since the decreasing photoperiod in autumn accelerates gonadal maturation (Billard *et al.*, 1978). Testicular maturation of underyearling precocious male masu salmon could be experimentally manipulated by changing the length of the light-dark photoperiod. Maturation was accelerated by changing the photoperiod from natural to short (8L16D) in June, and was delayed by keeping the photoperiod long (16L8D) from June (Amano *et al.*, 1994a). sGnRH mRNA levels in the VT and the POA increased when the fish spermiated; the activity increased in August in the 8L16D group, and in September in the 16L8D group, respectively. Moreover, the increase of sGnRH mRNA levels was in accordance with the increase of pituitary GTH II contents (Fig. 6). No significant changes in sGnRH mRNA levels in relation to gonadal maturation were observed in the OB and the TNG (Amano *et al.*, 1995b). These results indicate that sGnRH neurons in the VT and the POA are influenced by photoperiod, and are involved in the

regulation of gonadal maturation through GTH secretion.

(c) Changes in sGnRH mRNA expression induced by sex steroids

Accumulation of GTH in the pituitary can be stimulated by aromatizable androgen or estrogen in juvenile fish; this is a known positive feedback system (see Goos, 1987). The involvement of GnRH in this mechanism is speculated, because sex steroid administration increased the amount of GnRH in the hypothalamus of the European silver eel (Dufour *et al.*, 1985), and rainbow trout (Goos *et al.*, 1986). On the other hand, the stimulatory effects of testosterone on the expression of pituitary GTH IIβ gene was demonstrated *in vitro* in juvenile rainbow trout (Xiong *et al.*, 1993). Thus, whether steroids have direct actions on the pituitary, or act indirectly via GnRH was unclear.

We examined the effects of 17α-methyltestosterone (MT) on sGnRH mRNA expression in yearling masu salmon (Amano *et al.*, 1994b). Oral MT application markedly increased pituitary GTH IIβ, but not GTH Iβ concentrations in both sexes. In future precocious males, MT treatment further increased the number of neurons expressing sGnRH mRNA in the POA but not in the OB and the VT (Fig. 7), whereas sGnRH mRNA levels were not changed by MT in immature females. These results suggest that sGnRH is involved in the positive feedback system at least in future precocious males, and that the difference in the responsiveness of preoptic sGnRH neurons to MT is based on the maturational stage of the fish. The result that sGnRH mRNA levels in the POA was increased by the administration of MT in 2-year-old females which are just before the initiation of gonadal maturation supports this hypothesis (Amano *et al.*, 1997). Of note, studies on pubertal development have been conducted in a number of teleost fish species such as the rainbow trout (Gielen *et al.*, 1982; Goos *et al.*, 1986), the eel (Dufour *et al.*, 1985; Counis *et al.*, 1987), the platyfish (Schreibman *et al.*, 1986), and African catfish (Schulz *et al.*, 1994a,b). These studies suggest that sex steroids initiate and/or accelerate the development of the brain-pituitary-gonadal axis. Taken together, the response of preoptic sGnRH neurons to steroids is a necessary condition for gonadal maturation or the development of the brain-pituitary-gonadal axis.

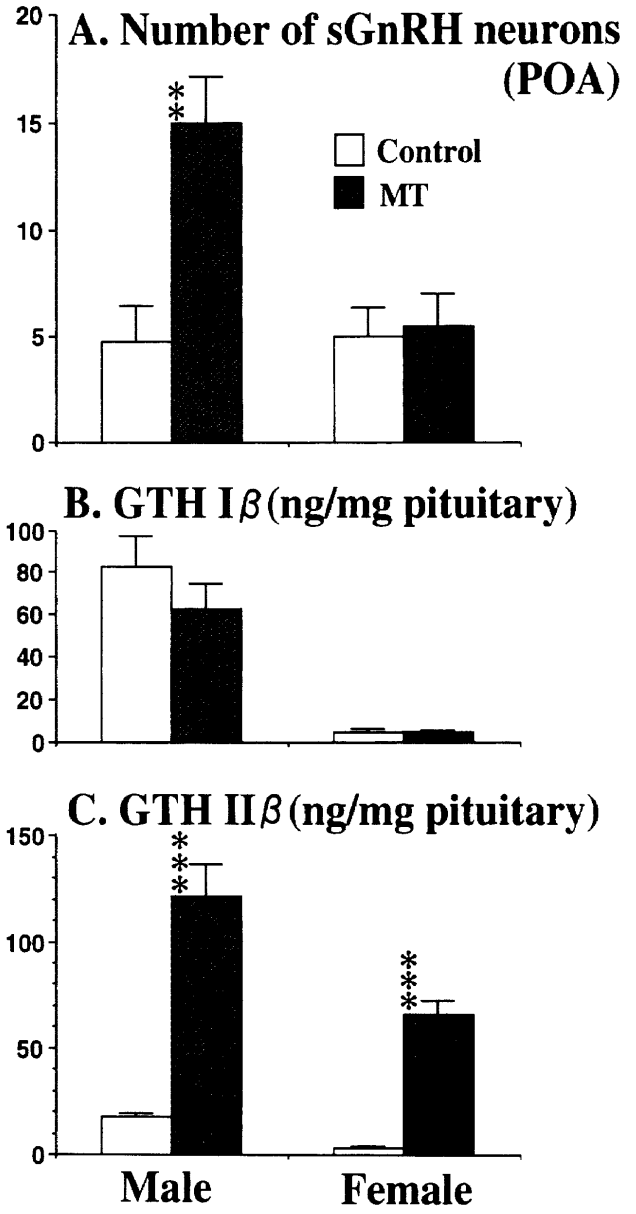

Fig. 7. Changes in (**A**) the number of neurons expressing sGnRH mRNA in the POA, (**B**) pituitary GTH Iβ concentrations, and (**C**) pituitary GTH IIβ concentrations in the control and the MT-treated groups. **($P<0.01$) and ***($p<0.001$) indicate the level of significant difference between the control and the MT-treated groups.

(d) Changes in sGnRH mRNA expression during seaward migration

sGnRH neurons in the ON and the OB may be involved in the seaward migration of salmonid fish, as the importance of olfactory functions during homing migration has been confirmed. Interestingly, sGnRH mRNA expression in cribriform ganglion in the ON increased during downstream migration in juvenile chum salmon (Parhar *et al.*, 1994). Meanwhile, changes of sGnRH-producing neurons in chum salmon during homing migration from coastal seas to the spawning ground of the maternal river was examined using ISH (Kudo *et al.*, 1996). sGnRH mRNA levels in the ON and the ON-OB were high in fish in the coastal seas, but activity decreased in the spawning ground. These results suggest that sGnRH neurons in the ON and probably some in the ON-OB may be involved in seaward migration.

(e) Existence of two differing precursor genes for sGnRH

Existence of two different genes for sGnRH have been demonstrated in sockeye salmon (Ashihara *et al.*, 1995). These authors found two different cDNAs for sGnRH precursors (pro sGnRH-I and pro sGnRH-II); pro sGnRH-I cDNA is 436 base pair long (short type) and pro sGnRH-II cDNA is 482 base pair long (long type). A question arising here is whether the two genes encoding sGnRH are expressed equally or differently. We are now attempting to examine the distribution of sGnRH neurons expressing short type and/or long type

sGnRH precursors in the sockeye salmon brain using ISH.

CONCLUSION

At present, nine GnRH molecules have been characterized throughout the vertebrates, and the existence of multiple forms of GnRH in the brain was observed also in teleost fish. All of the teleosts examined thus far have cGnRH-II, whereas the second form is either sGnRH, mGnRH, catfish GnRH, or seabream GnRH. Distribution of GnRH neurons was examined using immunocytochemistry. In general, sGnRH neurons were distributed from the ON to the POA or the hypothalamus, whereas cGnRH-II neurons were detected in the midbrain tegmentum. GnRH-ir fibers were distributed in various brain regions, indicating multiple neuroendocrine functions in the brain.

The brain GnRH contents were measured by RIA and related to gonadal maturation, although results are discordant. It was demonstrated that most of the sGnRH fibers in the brain originate from the TNG, and that sGnRH derived from the TNG is not essential for gonadal maturation.

Recent determination of the cDNA encoding GnRH precursors has enabled the investigation of GnRH synthetic activity using ISH. The distribution of GnRH ISH positive neurons was examined in several teleost fishes and the results corresponded to those obtained by immunocytochemical investigation. We examined sGnRH mRNA expression of salmonid fishes, and found that sGnRH neurons in the different brain regions have different functions according to their location.

The function of sGnRH in the brain of teleost fish, especially in salmonids, has been partly elucidated by use of RIA, immunocytochemistry and ISH. However, the function of cGnRH-II, which is distributed in almost all of the teleost fishes examined so far, remains unclear, except for the stimulation of GTH and GH release *in vitro*.

It has been proposed that GnRH-ir neurons are derived from the olfactory placode and migrate to the forebrain during prenatal development in the mouse (mGnRH; Schwanzel-Fukuda and Pfaff, 1989; Wray *et al.*, 1989), the chicken (probably cGnRH-I, Murakami *et al.*, 1991), and the newt, *Cynops pyrrhogaster* (probably mGnRH, Murakami *et al.*, 1992). In chum salmon and sockeye salmon, GnRH (probably sGnRH) neurons originate in the olfactory placode and then migrate into the brain along the ON (Chiba *et al.*, 1994; Parhar *et al.*, 1995). Recently, it has been reported that cGnRH-II are not olfactory placode origin in the axolotl, *Ambystoma mexicanum* (Northcutt and Muske, 1994). cGnRH-II may not originate from the olfactory placode in teleost fish as well. Studies on the ontogeny of the GnRH system may be helpful in understanding the function of GnRH in the brain.

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