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Molecular characterization and expression profiles of three GnRH forms in the brain and pituitary of adult chub mackerel (*Scomber japonicus*) maintained in captivity

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ABSTRACT

Gonadotropin-releasing hormone (GnRH) is a key neuroendocrine peptide involved in the reproduction of fish and other vertebrates. However, characterizing the involvement of GnRH in fish reproduction has been complicated by the discovery of multiple GnRH forms. In the present study, we isolated full-length cDNAs encoding three GnRH forms and analyzed seasonal changes in the concentrations of mRNA in the brain and corresponding peptides in the brain and pituitary, in relation to seasonal gonadal development of chub mackerel (*Scomber japonicus*). Chub mackerel sbGnRH, cGnRH-II, and sGnRH cDNAs encode 98, 85, and 90 deduced amino acids, respectively. In females, brain sbGnRH mRNA and peptide concentrations were significantly higher only during the post-spawning season (August); however, pituitary peptide concentrations were higher during late vitellogenesis (April) and the post-spawning season, in comparison to immature stage (November). In males, brain sbGnRH mRNA and pituitary peptide concentrations were higher during spermiation (April). No significant differences in cGnRH-II mRNA or peptide concentrations were found in either sex. Furthermore, in females, brain sGnRH mRNA concentrations did not vary significantly; however, corresponding peptide concentrations in the brain and pituitary were higher during late vitellogenesis and the post-spawning season, respectively. In males, only brain sGnRH mRNA concentrations were higher during the post-spawning season, with no significant change in peptide concentrations. This study quantified the seasonal expression changes of three GnRH mRNAs and peptides in both sexes of chub mackerel, and the present results combined with our previous immunocytochemical report indicates that sbGnRH form plays a dominant role in seasonal gonadal development.

1. Introduction

Fish represent the largest group of vertebrates and exhibit a wide range of reproductive strategies (Oliveira et al., 2005). The brain–pituitary–gonad (BPG) axis is a key neuroendocrine system involved in the reproductive processes, and gonadotropin-releasing hormone (GnRH) represents a key upstream signaling molecule in this system (Weltzien et al., 2004). Brain GnRH stimulates the synthesis and release of the pituitary gonadotropins (GtHs), follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which regulate the synthesis of sex steroids that are responsible for seasonal gonadal growth and maturation (Nagahama, 1994; Yaron et al., 2003). However, the fish brain expresses multiple GnRH forms, derived from distinct genes. This multiplicity has complicated our understanding of their roles in reproductive processes (Holland et al., 2001; Okubo and Nagahama, 2008). Presently, the GnRH family includes 30 different forms, representing 15 vertebrate and 15 invertebrate species; eight of these have been identified in fish (Roch et al., 2011). The GnRH forms in different species are classified as GnRH1, GnRH2, or GnRH3, based on phylogenetic analysis and neuroanatomical distribution (Fernald and White, 1999). GnRH1 is the hypophysiotropic form, with a distribution in the neuronal population of the preoptic area (POA) and hypothalamus. In fish, the GnRH1 forms include the mammalian form (mGnRH) and various fish-specific peptides such as seabream, medaka, whitefish, catfish, and herring GnRH (sbGnRH, mdGnRH, wfGnRH, cfGnRH, and hrGnRH, respectively). The GnRH2 form exists in the midbrain tegmentum region, and it is represented in all vertebrates examined to date by chicken GnRH-II (cGnRH-II). On the contrary, GnRH3 is a teleost-specific form (salmon GnRH, sGnRH) that is expressed in neuronal populations in the olfactory bulb, terminal nerve ganglion region, and POA (Kah et al., 2007; Lethimonier et al., 2004). For convenience, we refer to the various forms as 'GnRH forms' in the present study.

The chub mackerel (*Scomber japonicus*) is a marine pelagic fish, which belongs to the order Perciformes and family Scombridae. It is an important commercial fish throughout the tropical and temperate

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waters of the world and is widely distributed in the waters of Korea, China, Japan, and California (USA) (Collette, 2003; Hwang and Lee, 2005). In Japan, chub mackerel fishery has been managed by the total allowable catch (TAC) system since 1997. Aquaculture of chub mackerel commenced in southwestern Japan due to unpredictable yield of the wild fish. The high demand for live fish is currently addressed using young or adult fish caught from the wild (Matsuyama et al., 2005). This species has been suggested to be a potential scombroid fish for aquaculture, considering its high early growth potential and use in the tuna fishing industry as live bait (Mendiola et al., 2008). The wild-caught adult chub mackerels reared in sea pens and outdoor tanks undergo normal spermatogenesis and vitellogenesis (Matsuyama et al., 2005). This experimental system facilitates fish sampling at different gonadal growth stages to elucidate the role of key neuroendocrine hormones acting at the BPG axis, which are responsible for seasonal gonadal development. However, after the completion of vitellogenesis, female fish fail to undergo final oocyte maturation (FOM) or ovulation during spawning season (April–June) (Shiraishi et al., 2005). The main reason for the failure of FOM in captive stock seems to involve endocrinological dysfunction associated with a lack of spontaneous LH surge, which is typically orchestrated by the pituitary (Shiraishi et al., 2008a).

Our previous study found that the chub mackerel brain extracts contain peptides that are chromatographically and immunologically identical to sbGnRH, cGnRH-II, and sGnRH. Further, their neuronal distribution, as revealed using immunocytochemistry, showed that sbGnRH-immunoreactive cell bodies localized in the POA send their axonal projections to the pituitary (Selvaraj et al., 2009). However, teleosts expressing three GnRH transcripts in the brain exhibit differences in abundance of their corresponding peptides in the pituitary; with one or two forms show fluctuation in relation to seasonal gonadal development (Holland et al., 2001; Senthilkumaran et al., 1999). In addition, GnRH is regulated at the transcription, translation, and secretion levels to produce characteristic physiological effects (Nelson et al., 1998). Previous studies measured either mRNA concentrations in the brain and corresponding peptide concentrations in the pituitary or only peptide concentrations in the brain and pituitary to correlate their fluctuations in relation to seasonal gonadal development and maturation (Amano et al., 2008). In the present study, an attempt was made to measure both GnRH mRNA concentrations in the brain as well as their corresponding peptide concentrations in the brain and pituitary to clarify the GnRH form predominantly involved in the seasonal gonadal development of chub mackerel.

With this background, the present study was conducted with the following aims: (1) to characterize cDNAs encoding sbGnRH, cGnRH-II, and sGnRH in the brain of chub mackerel; and (2) to analyze the changes in expression of three forms of GnRH, as measured by mRNA concentrations in the brain and corresponding peptide concentrations in the brain and pituitary during different gonadal stages, using quantitative real-time polymerase chain reaction (qRT-PCR) and time-resolved fluoroimmunoassay (TR-FIA), respectively.

2. Materials and methods

2.1. Fish and tissue sampling

Adult chub mackerel were caught with a purse seine and reared in sea pens at a fish farm. The fish were reared under natural daylight and fed with commercial dry pellets (Higashimaru Co., Japan) twice per day. This fish stock was reared for one year at ambient temperature after its capture in November 2007, and experimental sampling was carried out from the same stock at different times of the year, representing different stages of reproductive development (Selvaraj et al., 2010). Female and male fish sampling was performed during the months of November 2008 (immature), early March (early vitellogenesis and late spermatogenesis), and late April 2009 (late vitellogenesis and spermiation), corresponding to gonadal growth periods (Shiraishi et al., 2008b). In addition, the sampling was performed during August 2009, corresponding to post-spawning period. During each sampling period, fish were transferred and stocked in 3-ton outdoor concrete tanks at the Tsuyazaki fishery laboratory of Kyushu University. Appropriate measures were taken to prevent handling stress to fish. The fish were maintained in tanks supplied with natural seawater and sampling was performed after one week of acclimatization. The water temperature at the time of sampling in November, March, April, and August was 20.6 °C, 11.9 °C, 15.8 °C, and 24.5 °C, respectively. At each sampling point, female and male fish (n = 12–14 for each sex) were sacrificed in accordance with the guidelines for animal experiments proposed by the Faculty of Agriculture and Graduate Study at Kyushu University and according to the laws (no. 105) and notifications (no. 6) of the Japanese government. All fish sampling was performed during the morning and early afternoon.

Body weight and gonad weight were measured to calculate the gonadosomatic index (GSI = gonad weight/body weight without gonads × 100). The brain and pituitary of each fish were removed following decapitation, snap-frozen in liquid nitrogen, and stored at −80 °C until further analysis. The midsection of each gonad from individual fish was fixed in Bouin's solution for gonadal histology. To analyze the changes in sbGnRH, cGnRH-II, and sGnRH mRNA in the whole brain and corresponding peptide levels in the whole brain

Table 1
Fork length, body weight, and gonadosomatic index (GSI) of the female and male chub mackerels used for GnRH mRNA and peptide analyses. Values are expressed as the mean ± SEM. Different characters represent significant differences ($P<0.05$) among months.

Analyses	Sex	Parameters	Sampling periods			
			November 2008	March 2009	April 2009	August 2009
GnRH mRNAs	Females	Fork length (cm)	33.8 ± 0.3^{a}	34.6 ± 0.3^{a}	33.6 ± 0.4^{a}	34.6 ± 0.2^{a}
		Body weight (g)	523 ± 16^{a}	566 ± 35^{a}	523 ± 24^{a}	523 ± 33^{a}
		GSI (%)	1.0 ± 0.0^{a}	1.4 ± 0.1^{a}	7.3 ± 1.4^{b}	4.1 ± 0.9^{ab}
		n	6	6	6	6
	Males	Fork length (cm)	33.1 ± 0.4^{a}	34.2 ± 0.3^{ab}	33.0 ± 0.3^{a}	35.0 ± 0.5^{b}
		Body weight (g)	489 ± 20^{a}	518 ± 22^{a}	513 ± 15^{a}	553 ± 37^{a}
		GSI (%)	0.3 ± 0.1^{a}	2.2 ± 0.5^{a}	12.0 ± 1.3^{b}	2.6 ± 1.0^{a}
		n	6	6	6	6
GnRH peptides	Females	Fork length (cm)	33.5 ± 0.1^{a}	33.8 ± 0.3^{a}	33.5 ± 0.5^{a}	34.6 ± 0.3^{a}
		Body weight (g)	496 ± 11^{a}	523 ± 23^{a}	522 ± 24^{a}	503 ± 32^{a}
		GSI (%)	0.8 ± 0.1^{a}	1.4 ± 0.1^{a}	6.7 ± 0.8^{b}	6.3 ± 0.9^{b}
		n	6	6	6	5
	Males	Fork length (cm)	33.0 ± 0.4^{a}	34.1 ± 0.6^{a}	33.3 ± 0.4^{a}	33.8 ± 0.3^{a}
		Body weight (g)	490 ± 16^{a}	552 ± 44^{a}	552 ± 29^{a}	479 ± 15^{a}
		GSI (%)	0.2 ± 0.0^{a}	2.2 ± 0.6^{a}	12.1 ± 0.9^{b}	1.2 ± 0.3^{a}
		n	5	7	6	7