Incomplete Development of the Brain-Pituitary-Gonadal Axis May Underlie the Delay in the Initiation of Precocious Maturation in Male Sockeye Salmon

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Precocious males first appear in the second year in sockeye salmon, whereas in masu salmon they appear in the first year. In underyearling male masu salmon, precocious maturation is accelerated under short photoperiod with an increase in salmon gonadotropin-releasing hormone (sGnRH) and pituitary gonadotropin subunit (GTH I β and GTH II β) contents, suggesting that the brain-pituitary-gonadal axis is already completed. In this study, the development of the brain-pituitary-gonadal axis in underyearling male sockeye salmon was investigated by manipulating photoperiod in order to clarify the relationship between its development and initiation of precocious maturation. Fish were divided into short (8L-16D) and long photoperiodic (16L-8D) groups in June and were reared for 4 months until October. sGnRH contents in the brain and pituitary, and GTH I β and GTH II β contents in the pituitary were measured. sGnRH contents in the hypothalamus and the pituitary, and GTH I β contents in the pituitary increased under short photoperiod, although gonadal maturation was not induced under either photoperiod. However, pituitary GTH II β contents did not increase in either photoperiodic group. These results suggest that in contrast to masu salmon, the brain-pituitary-gonadal axis is not completed in underyearling male sockeye salmon. This species-specific difference in the development of the brain-pituitary-gonadal axis may underlie differences in the initiation of precocious maturation.

Key words: precocious, salmon GnRH, GTH, sockeye salmon

It is well known that precocious maturation occurs in male salmonid fishes including Atlantic salmon *Salmo salar*,^{1,2)} amago salmon *Oncorhynchus rhodurus*,³⁾ masu salmon *Oncorhynchus masou*,⁴⁻⁶⁾ and sockeye salmon *Oncorhynchus nerka* (Amano *et al.*, unpublished data). However, the age at which fish mature precociously differs according to species. For example, most male masu salmon mature precociously in the first autumn, while precocious males first appear in the second summer in sockeye salmon landlocked in Japanese lakes. Although the cause of this species-specific difference in the initiation of precocious maturation is of much interest, fundamental mechanisms have not yet been elucidated.

Gonadal maturation in teleost fishes is regulated by the brain-pituitary-gonadal axis; gonadotropin-releasing hormone (GnRH) which is synthesized in the brain stimulates the synthesis and release of gonadotropin (GTH) in the pituitary, and the thus-released GTH stimulates steroid hormone secretion from the gonads.⁷⁾ Thus, there is a possibility that the periodic difference in the appearance of precocious maturation between masu salmon and sockeye salmon is due to differences in the development of the brain-pituitary-gonadal axis.

We have previously shown that the brain-pituitary-gonadal axis is already completed in underyearling male masu salmon; salmon GnRH (sGnRH) mRNA levels in the

brain and GTH II contents in the pituitary increased with the advancement of testicular maturation controlled experimentally by manipulating photoperiod.^{8,9)}

In the present study, we manipulated photoperiodic conditions in June (when photoperiod is longest) from natural to short (8L-16D) or long (16L-8D) photoperiod using underyearling male sockeye salmon to clarify the relationship between precocious maturation and the development of the brain-pituitary-gonadal axis. We examined testicular development and measured sGnRH contents in the brain and pituitary, and GTH subunit (GTH I β and GTH II β) contents in the pituitary.

Materials and Methods

Fish

Experiments were conducted at the Nikko Branch of the National Research Institute of Aquaculture, Nikko, Tochigi Prefecture. Sockeye salmon eggs were fertilized artificially in August 1988 and hatched in October 1988. The fish were reared in indoor tanks under natural photoperiod using spring water of constant temperature (9–10°C) before conducting experiments. Although the fish of both sexes matured at 3 years of age, some males precociously matured at 2 years of age. On June 7, 1989, 500 underyearling sockeye salmon of both sexes (mean body

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weight 8.0 g) were randomly selected from a stock and were divided into the following two categories (250 fish each): short photoperiod group (8L-16D; lights on 09:00–17:00), and long photoperiod group (16L-8D; lights on 04:00–20:00). Each group was transferred to a dark room shielded with black cloth. Photoperiod was manipulated using four 10-W fluorescent lamps (two lamps on each tank) and a timer. The fish were reared in round tanks (80 cm diameter and 300 l volume) using spring water of constant temperature (9–10°C) throughout the experiment.

Sampling

We sampled only male fish; data for females were omitted from analysis in this study. Tissues for hormone analyses were collected on June 7, August 8, August 23, September 13 and October 11. Sampling procedures were described by Amano *et al.*¹⁰⁾ At autopsy, fish were randomly selected and anesthetized in ethyl-*p*-aminobenzoate (0.05%). After measurements of body length (BL) and body weight (BW), brain (olfactory bulb, telencephalon including preoptic area, hypothalamus) and pituitary intended for the measurement of sGnRH and GTH (GTH I β and GTH II β) were removed and frozen on dry ice. The brain and pituitary tissues were stored at –80°C until extraction. Gonads were fixed with Bouin's fluid for 24 hours and then their weights were measured for determination of gonadosomatic index (GSI). Gonads were embedded in paraffin and sectioned at 5 μ m. The sections were stained with hematoxylin and eosin for histological observation.

GnRH and GTH RIAs

sGnRH contents in the brain and pituitary, and GTH I β and GTH II β contents in the pituitary were measured by RIA. Methods for the extraction and measurement of GnRH and GTH were as described in Okuzawa *et al.*¹¹⁾ and Amano *et al.*¹⁰⁾ It was ascertained by HPLC-RIA analysis that sockeye salmon brain contains sGnRH. Brain extracts of sockeye salmon showed displacement curves which were parallel to the curves for the sGnRH standard. Moreover, displacement curves for pituitary samples of sockeye salmon were parallel to the standard curves in both GTH I β and GTH II β RIA (data not shown).

Statistics

The Student's *t*-test, Cochran-Cox test, Duncan's new multiple range test and Kruskal-Wallis test were used for statistical analysis.

Results

Growth

BL was significantly larger in the 16L-8D group than in the 8L-16D group in early and late August (Fig. 1A). BW was significantly larger in the 16L-8D group than in the 8L-16D group in late August (Fig. 1B).

Gonadal Development

Precocious males did not appear throughout the experiment in either photoperiodic group; primary spermatogonia or secondary spermatogonia were observed in all individuals (data not shown). However, GSI showed wider distribution in the 8L-16D group than in the 16L-8D group

Fig. 1. Changes in body length (A) and body weight (B).

Closed and open circles represent the data of the 8L-16D and the 16L-8D group, respectively. Numbers beside each symbol indicate the number of fish employed. Each value is expressed as the mean (point) and the standard error (bar). * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$) indicate the levels of significant differences between the 8L-16D and the 16L-8D groups. In each group, means with differing letters differ significantly ($p < 0.05$).

in August and September. No remarkable changes in GSI were seen in either the photoperiodic group nor between the 8L-16D and the 16L-8D groups throughout the experiment (Fig. 2).

GTH I β and GTH II β Contents in the Pituitary

GTH I β contents in the pituitary increased significantly in early August in the 8L-16D group. GTH I β contents in the 8L-16D group were significantly higher than those in the 16L-8D group in early and late August and October (Fig. 3). GTH I β contents remained at low levels throughout the experiment in the 16L-8D group. In the 8L-16D group, GTH I β had a highly positive correlation with GSI only in September (correlation coefficient 0.9683, $p < 0.001$, $n = 9$). These positive correlations were not observed in the 16L-8D group throughout the experiment.

GTH II β contents in the pituitary were below the detectable limit (1.25 ng/pituitary) in all individuals throughout the experiment (data not shown).

sGnRH Contents in the Brain and Pituitary

sGnRH contents in the telencephalon tended to be higher in the 8L-16D group than in the 16L-8D group from late August, although the differences were not statistically significant (Fig. 4A). sGnRH contents in the hypothalamus also tended to be higher in the 8L-16D group, and were significantly higher in the 8L-16D group in September (Fig. 4B). sGnRH contents in the pituitary

Fig. 2. Changes in gonadosomatic index (GSI).

Data are shown for individual values, the mean and the standard error. Closed and open circles represent the data of the 8L-16D and the 16L-8D group, respectively. In each group, means with differing letters differ significantly ($p < 0.05$).

Fig. 3. Changes in pituitary GTH Iβ contents.

Closed and open circles represent the data of the 8L-16D and the 16L-8D group, respectively. Each value is expressed as the mean (point) and the standard error (bar). * ($p < 0.05$) indicates the levels of significant differences between the 8L-16D and the 16L-8D groups. In each group, means with differing letters differ significantly ($p < 0.05$).

were below the detectable limit (4.4 pg) in June and early August in all individuals. Levels became detectable and significantly higher in the 8L-16D group than in the 16L-8D group in September and October (Fig. 4C). Significant differences in sGnRH contents were not observed in the olfactory bulb between both photoperiodic groups at each sampling time (data not shown).

Discussion

Short photoperiodic manipulation increased levels of sGnRH (hypothalamus and pituitary) and GTH Iβ (pituitary), although precocious maturation was not induced in the present study. These results suggest that synthesis and storage of sGnRH increased under short photoperiod and then sGnRH stimulates GTH Iβ synthesis. It is reported that GTH I is involved in the early phases of spermatogene-

Fig. 4. Changes in sGnRH contents in the telencephalon (A), the hypothalamus (B), and the pituitary (C).

Closed and open circles represent the data of the 8L-16D and the 16L-8D group, respectively. Each value is expressed as the mean (point) and the standard error (bar). * ($p < 0.05$) and ** ($p < 0.01$) indicate the levels of significant differences between the 8L-16D and the 16L-8D groups. In each group, means with differing letters differ significantly ($p < 0.05$). Arrow indicates the detectable limit.

sis in salmonid fishes.^{12,13} Indeed, GTH Iβ was positively correlated with GSI in September in the short photoperiod group. However, in contrast to GTH Iβ, levels of pituitary GTH IIβ were not increased. This result suggests that synthesis and release of GTH II are required for gonadal maturation.

In underyearling male masu salmon, sGnRH mRNA levels in the brain, sGnRH and GTH (GTH Iβ and GTH IIβ) levels in the pituitary increased with the advancement of testicular maturation,^{8,9} suggesting that the brain-pituitary-gonadal axis was already completed. On the other hand, it is probable that the brain-pituitary-gonadal axis was not completed in male sockeye salmon, since increase

of GTH II levels and induction of testicular maturation were not observed. This species-specific difference in the development of the brain-pituitary-gonadal axis may underlie differences in the initiation of precocious maturation. If we had used yearling male sockeye salmon whose brain-pituitary-gonadal axis is considered to be completed, testicular maturation might have been accelerated by manipulating photoperiod.

There are some evidences suggesting retarded development of the sGnRH system in sockeye salmon compared to masu salmon. In underyearling male fish in June, sGnRH contents were lower in sockeye salmon than masu salmon. In sockeye salmon (mean BL: 8.8 cm, BW: 7.9 g), sGnRH contents in the telencephalon and the hypothalamus were 98 pg and 86 pg and sGnRH was below the detectable limits (4.4 pg) in the pituitary, whereas in masu salmon (mean BL: 8.0 cm, BW: 7.4 g), sGnRH contents in the telencephalon, the hypothalamus and the pituitary were 265 pg, 312 pg and 10 pg, respectively.⁸⁾ It is also suggested that the appearance of sGnRH-immunoreactive (ir) cell bodies and fibers as detected by immunocytochemistry is delayed in sockeye salmon compared to masu salmon. In sockeye salmon, sGnRH-ir cell bodies and fibers in the brain were first detected in the fish 10 months after hatching,¹⁴⁾ whereas in masu salmon, sGnRH-ir fibers were already detected in fish just after hatching and sGnRH-ir cell bodies were first detected in fish 5 months after fertilization.¹⁵⁾

BL was significantly larger in the long photoperiod group than in the short photoperiod group, suggesting that long photoperiod stimulates growth hormone (GH) secretion. This hypothesis is supported by the results in underyearling masu salmon that plasma GH levels increased after changing the photoperiod from short (8L-16D) to long (13L-11D).¹⁶⁾ Incidentally, sGnRH is known to stimulate not only GTH release but also GH release from the pituitary in goldfish *Carassius auratus*^{17,18)} and sockeye salmon (Amano et al., unpublished data). In masu salmon, the differentiation of precocious males may occur in May, when the photoperiod is long.¹⁹⁾ Taken together, it is possible that GH secreted under a long photoperiod has some role in initiating precocious maturation. This hypothesis is supported by the observations that body growth plays an important role in the differentiation of precocious and immature males in masu salmon; precocious males are larger in BL than immature males.^{4,5)}

In summary, it is suggested that, in contrast to masu salmon, the brain-pituitary-gonadal axis is not completed in underyearling male sockeye salmon. This species-specific difference in the development of the brain-pituitary-gonadal axis may underlie differences in the period of precocious maturation.

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