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**HOKKAIDO UNIVERSITY**
Changes of Two Types of Pituitary Gonadotrophs in Whitespotted Char, *Salvelinus leucomaenis*, During Gonadal Development*

Hiroshi UEDA**

Abstract

In the proximal pars distalis of the pituitary gland of male and female whitespotted char, *Salvelinus leucomaenis*, two distinct types of glandular cells, termed the globular and vesicular cells, were discerned from possible thyrotrophs and somatotrophs by their locality, staining property and ultrastructure. Changes of the two types of cells were examined light and electron microscopically during the course of first maturation and spawning of the fish.

A few globular cells with immature cytoplasmic granules were already in existence in the pituitaries of one-year old fish with ovaries at the early yolk vesicle stage or with testes at the spermatogonial stage. In harmony with the subsequent development of the gonads, the globular cells showed a notable increase both in number and in size, and displayed a degranulation and a dilation of endoplasmic reticulum, followed by some atrophic changes, at the time of spawning. By contrast, the vesicular cells, which were absent in the pituitaries of immature one-year old fish, made their appearance concurrently with the appearance of oocytes in the late yolk vesicle stage or of the first spermatocytes in the gonads. They showed little change during the period of advancing vitellogenesis and spermatogenesis, but became activated again, with the development of the Golgi apparatus and the accumulation of cytoplasmic granules, 3 months after spawning when ovarian oocytes were at the late yolk vesicle stage.

It was concluded that the two types of pituitary cells in the char were gonadotropic in function, each acting in different phases of gonadal development. Possible significance of the vesicular cells was discussed with respect to their implication in the initiation of vitellogenesis and spermatogenesis.

The problem as to whether salmonid fishes possess one or two types of gonadotropic cells in the pituitary gland is still controversial at present. Earlier light microscopic studies on sockeye salmon, *Oncorhynchus nerka*, suggested the presence of two types of gonadotrophs1,2, which was later confirmed by electron microscopy3, while some authors could find only one cell type in the same species of salmonids4,5. A similar discrepancy has occurred in the results in observations of the pituitary gonadotrophs of chum salmon, *O. keta*6,7. In coho salmon, *O. kisutch*8, and masu salmon, *O. masou*9, the possible existence of different types of gonadotrophs was stressed by light and electron microscopic studies, respectively.

Recently, Olivereau9,10 presented cytological and cytochemical evidence for

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* Contribution No. 12 from the Nanae Fish-Culture Experimental Station, Faculty of Fisheries, Hokkaido University.
** Laboratory of Fresh-Water Fish-Culture, Faculty of Fisheries, Hokkaido University
two distinct types of gonadotrophs in the pituitary gland of Atlantic salmon, *Salmo salar*, rainbow trout, *S. gairdneri*, and brown trout, *S. fario*. On the contrary, Ekengren *et al.* described, in the Atlantic salmon, a single type of pituitary gonadotrophs which was capable of assuming diverse ultrastructural features during the reproductive cycle. A similar view was expressed by Peute *et al.* about pituitary gonadotrophs of the rainbow trout.

To solve the problem in question, it seems effective to carry out careful studies, particularly from ultrastructural viewpoints, on the changes of pituitary cells from the beginning of gonadal development to full maturation in fishes. Several salmonid species such as the rainbow trout and whitespotted char, *Salvelinus leucomaenis*, which are easy to culture and breed in the pond, are considered to be suitable material for such a purpose. Moreover, only a few brief reports have been concerned so far with the histology of the pituitary gland of fish belonging to the genus *Salvelinus*. The present study was therefore conducted in order to observe ultrastructural changes of the pituitary gonadotrophs of the whitespotted char, *Salvelinus leucomaenis*, using pond-cultured fish at various stages of their gonadal development.

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**Material and Methods**

The whitespotted char, *Salvelinus leucomaenis*, used in this study were obtained from stocks of fish which had been cultured, continuously following hatching, in outdoor ponds at the Nanae Fish-Culture Experimental Station, Hokkaido University. Two-year old females, 20.5–36.5 cm in body length and 130–620 g in body weight, were sampled, 3 fish at a time, monthly from October 1977 to April 1978. In addition, a total of 39 one-year old fish of both sexes, 16.4–29.4 cm in body length and 65–300 g in body weight, were also collected during the months from April 1978 to September 1978.

They were killed by decapitation and their pituitary glands were immediately removed. Some of the glands were fixed with Bouin-Holland-sublimate, cut at 6 μm in thickness and stained with alcian blue-PAS-orange G. Others were prefixed with Karnovsky’s glutaraldehyde-paraformaldehyde mixture in 0.2M cacodylate buffer (pH 7.4) for about 3 hours at room temperature, postfixed in 1% osmium tetroxide in the same buffer for about 2 hours at 4°C, and embedded in Epon. Ultrathin sections stained double with uranyl acetate and lead citrate were observed with a Hitachi HU–12 electron microscope. Parallel sections of about 1 μm thick were stained with methylene blue for light microscopic comparison.
Results

The general morphology of the pituitary gland and the staining property of its constituent cells in the whitespotted char are very similar to those in the masu salmon\(^9\). Four types of glandular cells, one consists of acidophils and the other three of basophils, are present in the proximal pars distalis (PPD) of the pituitary gland of the fish. The acidophilic cells, possibly somatotrophs, are distributed evenly throughout the PPD. Basophilic cells of one type encountered in the dorsal region of the PPD show a weak affinity to both alcian blue and PAS. The cells are distinguished ultrastructurally by cytoplasmic granules, 100–200 nm in size, which are the smallest among those found in all granulated cells existing in the PPD. They are similar in this respect to thyrotophs identified in the pituitary of several other salmonid fishes\(^7\).

Of the other two types of basophils, those localized in the central and ventral regions of the PPD are stained intensely with both alcian blue and PAS. The cells are marked by having a few large globules, 800–1500 nm in diameter, along with numerous small granules, 200–400 nm in diameter, in the cytoplasm. The cells are thus designated as the globular cells in this paper. The rough endoplasmic reticulum of the cells is usually lamellar or irregularly vesicular in form, and contains electron lucent material in maturing fish of both sexes. Basophils of the remaining type distributed in the central and dorsal parts of the PPD are strongly positive to PAS but are almost negative to alcian blue. These, in adult male and female fish, are conspicuous ultrastructurally, on account of their rough endoplasmic reticulum constructed from numerous vesicular cisternae with material of low electron density. They are termed the vesicular cells hereafter. The cells also contain large globules, 500–800 nm in size, and small granules, 100–300 nm in size, in the cytoplasm, but these granular inclusions are relatively fewer in number and smaller in size than those of the globular cells.

Changes of putative gonadotrophs in females

One-year old females killed in April and May had ovaries with many oocytes in the early yolk vesicle stage. In these fish, a small number of alcian blue- and PAS-positive cells, which were rich in cytoplasmic granules, were distributed exclusively in the central parts of the PPD. The cells were mostly oval or elongate in shape with an oval shaped nucleus (Fig. 1). Ultrastructurally, the cells contained many small, electron dense granules of 150–350 nm in size and a few large, less electron dense globules of 400–1000 nm in size (Fig. 9). The rough endoplasmic reticulum was composed of flat or small vesicular cisternae with electron lucent contents. The Golgi apparatus was moderately active. Mitochondria were a few in number and small in size. These cytological features of the cells appeared to correspond to those of the globular cells. No typical cells of the vesicular type could be detected in one-year old fish examined in this period.

In June, ovaries of yearling females had oocytes in the late yolk vesicle stage. The globular cells were observed to be almost the same in features as those observed in the preceding months. In these fish, however, some cells with clear vacuoles in the cytoplasm appeared in the dorsal and central parts of the PPD. In these cells, granular inclusions were scarcely present in the cytoplasm, and the
Figs. 1–8. Light microscopic pictures of Epon-embedded, methylene blue-stained sections of the pituitary gland of female (Figs. 1–6) and male whitespotted char (Figs. 7 and 8), sampled in May (Fig. 1), June (Fig. 7), July (Fig. 2), September (Figs. 3 and 8), October (Fig. 4), November (Fig. 5) and January (Fig. 6). g, globular cell; n, non-granulated cell; NH, neurohypophysis; s, somatotroph; t, thyrotroph; v, vesicular cell. × 1100.
UEDA: Pituitary gonadotrophs of whitespotted char

Figs. 9 and 10. Electron micrographs of globular cells (g-GTH) and a vesicular cell (v-GTH) in the pituitaries of female whitespotted char sampled in May (Fig. 9) and June (Fig. 10). G, Golgi apparatus; LG, large globule; M, mitochondrion; rER, rough endoplasmic reticulum; SG, small granule; TSH, thyrotroph; V, vesicular cisterna of the rough endoplasmic reticulum. Scale, 1 μm.
Figs. 11 and 12. Electron micrographs of globular cells (g-GTH) and a vesicular cell (v-GTH) in the pituitaries of female whitespotted char sampled in July (Fig. 11) and September (Fig. 12). G, Golgi apparatus; LG, large globule; M, mitochondrion; NH, neurohypophysis; NGC, non-granulated cell; rER, rough endoplasmic reticulum; SG, small granule; STH, somatotroph; V, vesicular cisterna of the rough endoplasmic reticulum. Scale, 1 μm.
rough endoplasmic reticulum was composed of rounded vesicular cisternae (Fig. 10). These were thought to be vesicular cells that had differentiated, during that stage of the life-cycle, presumably from non-granulated cells preexisting in the PPD.

In yearling females collected in July, ovaries had many oocytes in the primary yolk stage. In these fish, a considerable number of vesicular cells were found in the dorsal and central regions of the PPD. These cells were rich in cytoplasm which was seen to contain many clear vacuoles by light microscopy (Fig. 2). Ultrastructurally, the cytoplasmic vacuoles coincided with rounded vesicles of the rough endoplasmic reticulum which packed the cytoplasm extensively. The dilated vesicles always contained material of low electron density. Small granules of 100-300 nm in size were gathered in groups, and large globules of 500-800 nm in size were few in number in these cells (Fig. 11). Well-developed Golgi apparatus and rod-shaped mitochondria appeared in the juxtanuclear region. The globular cells in these fish did not show any prominent changes in their structural aspects in comparison with those observed during the previous months.

In yearling females sampled in August and September, ovaries were filled with many oocytes advancing to the secondary and tertiary yolk stages. In the pituitary gland of these fish, the globular cells were observed to have increased in number and in size (Fig. 3). Moreover, a few cells of the globular cell type also existed among prolactin cells localized in the rostral pars distalis. In the cytoplasm of the globular cells in the PPD, small granules evidently increased in size attaining 200-400 nm (Fig. 12). Large globules also increased in size, measuring 800-1500 nm. Cisternae of the rough endoplasmic reticulum were dilated to various extents and contained electron lucent material. The Golgi apparatus was highly active, and sometimes immature granules were present within the Golgi field (Fig. 12). On the other hand, no notable ultrastructural change was observed in the vesicular cells when compared with those found in yearling females collected in July. Dilated vesicles characteristic of the cells were relatively uniform in size, and both small granules and large globules were fewer in number than those of the globular cells of the same period.

In October, the fish were at the peak of the spawning period. In the pituitaries of females with ovulated eggs in their coelomic cavity, the globular cells displayed remarkable changes (Figs. 4 and 13). Small granules in these cells were clearly diminished in number, while large globules appeared to remain unchanged in number but were seen to become more electron dense. Cisternae of the rough endoplasmic reticulum were dilated extensively so that they occupied most of the cytoplasm (Fig. 13). On the other hand, significant changes were barely detectable in the vesicular cells of females at the spawning stage by light and electron microscopy.

Females killed in November and December had ovaries with many postovulatory follicles and yolkless oocytes. In these fish, the globular cells displayed a degenerative feature (Figs. 5 and 14). The nucleus of the cells was quite irregular in shape and was unusually electron dense. Large globules increased in number and were observed to coalesce with each other, becoming irregular in shape (Fig. 14). On the other hand, the vesicular cells in these fish did not exhibit such a pycnotic
Figs. 13 and 14. Electron micrographs of globular cells (g-GTH) and a somatotroph (STH) in the pituitaries of female whitespotted char sampled in October (Fig. 13) and November (Fig. 14). G, Golgi apparatus; LG, large globule; M, mitochondrion; rER, rough endoplasmic reticulum; SG, small granule. Scale, 1 μm.

change, but were almost similar in structural features to those observed in October.

In January, three months after the spawning period, the ovary of 2-year old females had many oocytes of the yolk vesicle stage. The vesicular cells of these
Fig. 15 and 16. Electron micrographs of globular cells (g-GTH) and a vesicular cell (v-GTH) in the pituitary of female whitespotted char sampled in January. G, Golgi apparatus; LG, large globule; rER, rough endoplasmic reticulum; SG, small granule; STH, somatotroph; V, vesicular cisterna of the rough endoplasmic reticulum. Scale, 1 μm.
fish were seen to be larger in size than before (Fig. 6). In these cells, rounded cisternae of the rough endoplasmic reticulum were rather uneven in size, and some of them were atypically dilated (Fig. 15). Small granules were relatively abundant and existed in groups among the dilated vesicles, while large globules were scarcely detected in these cells. Well-developed Golgi apparatus consisting of minute vesicles and a stack of flattened lamellae were notable (Fig. 15). On the other hand, although some of the globular cells in January still retained a degenerative aspect, others became regranulated with small granules of 150–350 nm in size in the cytoplasm. A few large globules of 400–1000 nm were also present in these cells. The rough endoplasmic reticulum consisted of flat or small vesicular cisternae, and the Golgi apparatus appeared to be moderately active in these cells (Fig. 16).

During the ensuing months leading up to April, ovarian oocytes of 2-year old fish were continuously in the yolk vesicle stage. The vesicular cells regained their typical cytological aspects in February: rounded vesicles of the rough endoplasmic reticulum were uniform in size and small granules were fewer in number than those found in January. They stayed almost unchanged during the successive months. No significant alterations were observed also in the globular cells during that period.

Changes of putative gonadotrophs in males

One-year old males sampled in April and May had immature testes with spermatogonia only. Besides thyrotrophs and somatotrophs, there were only a few globular cells in the central part of the PPD, as was the case for the PPD of females of the same age.

In yearling males collected in June, a small number of first spermatoocyte cysts were present in their testes together with many spermatogonia. In the pituitaries of these fish, a considerable number of the vesicular cells were distributed in the dorsal and central parts of the PPD (Fig. 7). Rounded cisternae of the rough endoplasmic reticulum in these cells were evidently larger in size than those in the cells of females sampled in July (Fig. 17, compare with Fig. 11). Small granules of 100–300 nm in size and large globules of 500–800 nm in size were few in number in these cells. The globular cells in these fish were very similar in structural features to those observed in previous months.

During the subsequent course of testicular development, the vesicular cells displayed a pronounced decrease in the size of rounded cisternae of the rough endoplasmic reticulum, but small granules and large globules were almost unchanged in number (Fig. 18). In contrast, the globular cells began to exhibit a highly active feature in harmony with gonadal development. In August and September, a large amount of spermatozoa had accumulated in the widely expanded lumina of the testicular lobules in testes. Small granules in these cells were clearly increased in number (Fig. 8). Large globules were also increased in number, but some of them were decreased in electron density. The rough endoplasmic reticulum was extended to various degrees. The Golgi apparatus was quite active in the formation of immature granules (Fig. 18).
UEDA: Pituitary gonadotrophs of whitespotted char.

Figs. 17 and 18. Electron micrographs of globular cells (g-GTH) and vesicular cells (v-GTH) in the pituitaries of male whitespotted char sampled in June (Fig. 17) and September (Fig. 18). G, Golgi apparatus; LG, large globule; M, mitochondrion; rER, rough endoplasmic reticulum; SG, small granule; STH, somatotroph; V, vesicular cisterna of the rough endoplasmic reticulum. Scale, 1 μm.
Discussion

Light microscopic findings revealing the duality of pituitary gonadotrophs have been obtained in various salmonid fishes such as coho salmon, Oncorhynchus kisutch, Atlantic salmon, Salmo salar, rainbow trout, S. gairdneri, and brown trout, S. fario. These findings seem to be supported by previous ultrastructural studies on sockeye salmon, Oncorhynchus nerka, and masu salmon, O. masou. In whitespotted char, Salvelinus leucomaenis, two cell types in the PPD of the pituitary gland were determined to have light and electron microscopic characteristics similar to those identified as distinct types of gonadotrophs in other salmonids mentioned above. They were evidently different from possible somatotrophs and thyrotrophs in their ultrastructure as well as in their staining properties and localization in the PPD.

It seems certain that one of the cell types, designated as globular cells in this paper, is gonadotropic in function. The globular cells displayed a notable increase in size and in number in harmony with the progressive maturation of the gonad in the whitespotted char. In the latter phase of gonadal maturation, they came to be distributed in the rostral pars distalis of the pituitary, as was also the case for chum salmon, Oncorhynchus keta, the Atlantic salmon, and the masu salmon. Moreover, the cells conspicuously revealed a depletion of secretory granules, extensive dilation of the endoplasmic reticulum, and subsequent pycnotic changes of the nucleus in the female char following ovulation. The globular cells may secrete a gonadotropin which promote ovulatory changes of the ovary in the whitespotted char as they do in many other teleost fishes.

Pituitary cells of the other type, found in the char pituitaries which were termed the vesicular cells in this paper, were characterized by the presence of uniformly rounded cisternae of the rough endoplasmic reticulum throughout the cytoplasm. Their granular contents were always much fewer in number and smaller in size than those of the globular cells. The vesicular cells were clearly demarcated from the globular cells in the char of both sexes at every stage of gonadal development by these characteristics, except in immature yearling fish in which the former cell type was completely absent in the pituitaries.

In the rainbow trout, Peute et al. observed pituitary cells, which were similar in ultrastructure to the vesicular cells of the char, in varying amounts mixed with cells of the globular type depending on the time of the year and the sex of the fish. They concluded that gonadotrophs of a single type present in the trout pituitaries asynchronously changed their activities, from the globular to the cisternal (vesicular) stage, and vice versa, in the ultrastructural sense, during the reproductive cycle. Ekengren et al. also reached the same conclusion in their ultrastructural study on the Atlantic salmon. Peute et al., in particular, emphasized that some cells were ultrastructurally intermediate between the two stages, and that the cells of the cisternal stage were scarcely present in some months of the year. In the whitespotted char, however, no typical intermediate cell types were detectable ultrastructurally or light microscopically in the pituitaries examined in the present study. Moreover, both types of cells were evidently present, without showing notable change in their relative amount, in the pituitaries after the yearling fish had commenced vitellogenesis or spermatocyte formation.
In the pituitaries of immature, yearling whitespotted chars of both sexes, cells of the globular type with immature cytoplasmic granules existed in the PPD, while those of the vesicular type were indiscernible in that stage of gonadal immaturity. The vesicular cells made their first appearance in the PPD of the fish concurrently with the appearance of the sign of exogenous vitellogenesis in ovarian oocytes or with the formation of spermatocytes in the testes of the fish. It has been reported that in the Atlantic salmon possible gonadotrophs of the vesicular type become apparent in the fish caught in the sea when the gonadosomatic index was still very low.  

In the rainbow trout, the predominant occurrence of pituitary gonadotrophs of the vesicular stage was also correlated with vitellogenesis in females and spermatocyte formation in males. In the trout, however, they were observed to be shifted into the globular stage by gradual regranulation toward the spawning season, leading to absolute predominance of cells of the latter stage. By contrast, in the char, the vesicular cells did not show any conspicuous change during the period of advancing vitellogenesis and spermatogenesis, or even at the time of ovulation which accompanied remarkable modifications of the globular cells. The vesicular cells appeared to become active again, with the development of the Golgi apparatus and the accumulation of cytoplasmic granules, 3 months after spawning before the initiation of exogenous vitellogenesis in ovarian oocytes.

It may be concluded from the above-described results that the vesicular cells are the second pituitary gonadotrophs which, in concert with the globular cells, share in the control of gonadal activities of the whitespotted char. This possibility seems also to have been established by previous experimental studies in local, Misgurnus anguillicaudatus, which have two distinct types of pituitary gonadotrophs with similar ultrastructural characteristics to those of the char. While the globular cells exhibit noticeable changes at the time of spawning, the vesicular gonadotrophs are conspicuous for the lack of such an acute change throughout the reproductive cycle, the peak of their activity coinciding with the initiation of vitellogenesis and of spermatogenesis (Ueda, unpublished). It has been suggested that, also in the sockeye salmon, pituitary gonadotrophs of a vesicular cell type may have their essential gonadotropic potency in early stages of gonadal development.

One of the possible explanations of this phenomenon may be that, once vitellogenesis has been triggered by a secretion of the vesicular cells, the subsequent course of vitellogenesis extended over a long period of time depends on only a rather stable maintenance of the function of the cells. The globular cells may also have an indirect action on vitellogenesis presumably through stimulation of the secretion of estrogen which has been shown to increase in the blood plasma during the course of vitellogenesis. Activation of the vesicular cells might be under the influence of estrogen (Ueda and Takahashi, in press).

The precise roles played by the vesicular cells in testicular development of the char remain to be studied.
References


UEDA: Pituitary gonadotrophs of whitespotted char

