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Suppression of Prolactin Release *in vitro* from the Rainbow Trout Pituitary, with Special Reference to the Structural Arrangement of the Pituitary Cells

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ABSTRACT—In teleosts, prolactin (PRL) cells are localized in rostral region of the pars distalis (PD) forming clusters or follicles. The present study was undertaken to examine whether interactions resulting from the arrangement of PRL cells might be involved in the regulation of PRL release. To this end, the release of PRL from the rainbow trout (*Oncorhynchus mykiss*) and tilapia (*Oreochromis mossambicus*) was compared under three different conditions of incubation: 1) the organ culture of PRL cells in intact PD; 2) the incubation of individual PD cells that were dispersed and subsequently attached to the culture plate; 3) the incubation of PD cells that had been allowed to aggregate after dispersion. For the trout, PRL release from the dispersed cells was greater than that from either the organ-cultured PD or the cell aggregates. For the tilapia, by contrast, the release of PRL from dispersed cells was similar to that observed during the incubation of either the organ-cultured PD or the cell aggregates. In both the trout and tilapia, growth hormone (GH) cells form clusters in the proximal PD. For both species, the release of GH from the dispersed cells was similar to that from either the organ-cultured PD or the cell aggregates. For the trout, but not the tilapia, it would appear that the close association of PRL cells in pituitary follicles has considerable importance in establishing basal hormone release. The release of newly synthesized (pulse-labeled) PRL from dispersed trout PRL cells was reduced when the incubation medium was conditioned by previous incubation of the trout PD. Nevertheless, the release of newly synthesized PRL was not diminished by the addition of salmon PRL to the incubation medium. Taken together, our findings suggest that, for the trout, the suppression of PRL release from the PD and cell aggregates was mediated through (an) inhibitory factor(s) within the PD other than PRL itself.

INTRODUCTION

Teleost fish are unique among vertebrates in that the distal lobe of adenohypophysis (pars distalis, PD) is divisible topographically into rostral and proximal regions on the basis of the distinctive structure and arrangement of cell types. Among lower teleosts such as salmonids and eels, prolactin (PRL) cells are arranged into follicles in the rostral region of PD (RPD). A similar arrangement is also found in Chondrostei and Holostei [3, 13]. In more derived teleost fishes, however, PRL cells are typically organized into a nearly homogeneous mass in the RPD [3, 13]. Growth hormone (GH) and PRL are members of the same hormone family and likely to be derived from a common ancestral molecule [4]. In contrast to PRL cells, GH cells are generally localized in proximal region of PD (PPD), forming clusters of the cells in most species [3, 13]. The arrangement of PRL cells into discrete assemblages in the teleost RPD, that is clusters or follicles, led us to question whether this close arrangement itself or the opportunity it provided for paracrine interaction might be important in the regulation of PRL cell in these fishes. We choose to address this question using the PRL cells from two different species. These were the relatively primitive the rainbow trout, *Oncorhynchus mykiss*, and the highly derived the tilapia, *Oreochromis mossambicus*.

The tilapia produces two clearly distinct PRLs, PRL_{177} and PRL_{188} [26]; the former contains 177, and the latter 188 amino acid residues, of which only 69% are identical [37]. In salmonids, the chum salmon, *Oncorhynchus keta*, produces two quite similar PRL molecules that differ by only 4 amino acids while 98% are identical [38]. It is assumed that the two molecules have recently diverged and have resulted from the tetraploid genetic condition found in salmonids [23]. On the other hand, only one form of cDNA encoding PRL was found in the rainbow trout, suggesting that one form of PRL is clearly predominant over the other [18].

Organ-cultured tilapia pituitary releases large amounts of the two PRLs for several weeks [26]. In several teleost species including the tilapia, the dominant control over PRL release appears to be inhibitory while that of GH stimulatory [22]. On the other hand, PRL release from organ-cultured pituitary of the chum salmon and the rainbow trout (*O. mykiss*) as well as the Japanese eel (*Anguilla japonica*) was much less than GH release [1, 28, 29, 35, 36]. These results suggest that the control of PRL release from salmon and eel pituitaries, in which PRL cells are arranged as follicles, is different from that in the other teleost fishes.

In order to examine the association that might exist between the activity of PRL cells and their structural arrangement, we compared the magnitude of spontaneous hormone release from the dispersed cells attached to the culture plate

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with that from either organ-cultured PD or from the cell aggregates reconstituted from the dispersed PD cells in the trout and tilapia.

MATERIALS AND METHODS

Animals

Immature rainbow trout (*Oncorhynchus mykiss*), weighing 80–110 g, were obtained from a commercial source in Tokyo, and were reared in freshwater aquaria at the Ocean Research Institute of the University of Tokyo at 15°C for more than 2 weeks. They were fed commercial dry diet (Oriental, Chiba). Tilapia (*Oreochromis mossambicus*), weighing 200–400 g, were obtained from brackish water ditches at the AmOrient Aquaculture facility in Oahu, Hawaii. They were reared at the Hawaii Institute of Marine Biology of the University of Hawaii in fresh water at 25°C for more than 2 weeks. They were fed commercial dry diet (Purina Mills, Missouri).

Organ culture

The trout pituitary was removed after decapitation and placed in Ca^{2+}- and Mg^{2+}-free Hanks' balanced salt solution (Gibco Laboratories, New York) buffered with 25 mM HEPES and 18 mM $NaHCO_3$ to pH 7.4 and supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), and fungizone (0.25 μg/ml) (Gibco Laboratories). The osmotic pressure was 280–290 mosmol, isotonic to the trout plasma in fresh water. Tilapia pituitaries were held in the medium identical to that used for the trout except for the osmotic pressure, which was adjusted to 320–340 mosmol, isotonic to the tilapia plasma in fresh water by addition of NaCl. The pars distalis (PD) of the pituitary was dissected from the pituitary under dissecting microscope, and incubated in a well of a 96-well plate containing 200 μl Eagle's minimum essential medium (MEM; Nissui, Tokyo) with Earle's salt supplemented with kanamycin (60 μg/ml) and 10% fetal bovine serum (Gibco Laboratories). The medium osmotic pressure for the trout was 280–290 mosmol, and that for the tilapia was 320–340 mosmol. They were incubated for 1, 4 or 7 days at 15°C for the trout and at 25°C for the tilapia under an atmosphere of 95% O_2/5% CO_2. For determination of hormone release, the incubation media and the PD were stored at −80°C.

Cell dispersion

The trout and tilapia PD were dissected as described above. After washing with Hanks' balanced salt solution, the PD was minced into 0.2–0.5 mm blocks with a razor blade. The fragments were transferred to a siliconized suspension culture flask (Wheaton Scientific, New Jersey) filled with 10 ml Hanks' balanced salt solution containing 0.1 mM $CaCl_2$, 25 mg collagenase (Sigma, St. Louis), and 10 μg DNase I (Sigma). The mixture was stirred at 80 rpm and incubated at 20°C for 2 hr. The fragments were then washed with Hanks' balanced salt solution, and were dispersed mechanically by gentle suction and extrusion with a plastic transfer pipette. Dispersed cells were filtered through 37-μm nylon mesh, and harvested by centrifugation at 200×g for 10 min. Harvested cells were then resuspended in Eagle's MEM with Earle's salt supplemented with kanamycin (60μg/ml) and 10% fetal bovine serum (Gibco Laboratories). The medium osmotic pressure for the trout was 280–290 mosmol, and that for the tilapia was 320–340 mosmol. The cell yield and viability were assessed by counting in a hemocytometer in the presence of trypan blue (viability>90%; yield=2×10^5 cells/PD both in the trout and the tilapia).

Cell culture

The cells suspended in 200 μl MEM were seeded onto individual wells of a 96-well plate, and were preincubated for 2 days at 15°C for the trout and 25°C for the tilapia under an atmosphere of 95% O_2/5% CO_2. They were incubated further for 1, 4 or 7 days. The number of the cells attached on the plate after the preincubation was 2×10^4 cells/well. The incubation media were collected and were stored at −80°C for later determination of hormone release. The cells attached to the plate were resuspended with 10 mM phosphate buffer saline (PBS, pH 7.4) containing 0.1 mM $CaCl_2$, 25 mg collagenase. After counting the number with a hemocytometer (The yield was about 98% in all the experiments), the cells were harvested by centrifugation at 200×g for 10 min and were stored at −80°C.

Culture of the cell aggregates

The suspended cells of the PD were seeded at a density of 5×10^5 cells/ml MEM in a siliconized glass vial (20 mm in diameter). The vials were subjected to continuous gyratory shaking at 60 rpm at 15°C for the trout and 25°C for the tilapia under an atmosphere of 95% O_2/5% CO_2.

Radioimmunoassays

The organ-cultured PD, the dispersed cells, and the cell aggregates were sonicated in 10 mM PBS (pH 7.4) containing 0.1% Triton X-100 (pH 7.3). Prolactin and GH in the culture media and the tissue, the aggregate, or the dispersed cells were measured by specific radioimmunoassays for salmon PRL and GH [5, 12] or those for tilapia PRLs and GH [2]. The proportion of hormone-secreting cells to the total volume of organ-cultured PD was different from that of the dispersed cells and the cell aggregates, since the organ-cultured PD contained nerve fibers and connective tissue. In order to compare hormone release between each methods of incubation, therefore, released hormone into the medium was calculated as the percent fraction of the total hormone present in both the medium and the tissue (% release), providing relative hormone release standardized by the total content of specific hormone in each of samples.

Scanning electron microscope

The cell aggregates cultured for 4 days were fixed with 4% paraformaldehyde in 50 mM phosphate buffer (pH 7.4) for 2 hr at room temperature. They were dehydrated with ethanol, and were freeze-dried with t-buthyl alcohol by a JFD-300 freeze-drying device (JEOL, Tokyo). Then, they were coated by a JFC-1100 ion sputter coating device (JEOL), and observed by an ALPHA-25A scanning electron microscope (Akashi, Tokyo).

Immunocytochemistry

After washing with 10 mM PBS (pH 7.4), the cell aggregates were placed into a mixture of 4% paraformaldehyde and 1% picric acid in 50 mM phosphate buffer (pH 7.4) at room temperature for 1 hr. After fixation, they were dehydrated through graded ethanols and were embedded in paraplast (Monoject, St. Louis). Serial sections were cut at 3 μm and mounted on gelatinized slides. The immunocytochemical staining was performed by the avidin-biotin-peroxidase complex (ABC) method using a Vectastain ABC kit (Vector, California). For the trout, the same antisera employed in the previous study were used [36]. Anti-salmon PRL (LMH) was diluted 1:64K with 10 mM PBS (pH 7.4) containing 0.5% bovine serum albumin (BSA), and anti-salmon GH (AS9-2) was diluted 1:16K. For the tilapia, the same antisera as used by Ayson *et al.* [2]

for tilapia PRL_{188} and GH were used. Previous observations showed the co-localization of the two PRLs in the same cells of the tilapia pituitary [2, 21, 27]. Anti-tilapia PRL_{188} (P188-1-3) and anti-tilapia GH (G-4-4) were diluted 1 : 4K with 10 mM PBS (pH 7.4) containing 0.5% BSA. The specificity of immunoreactivity was tested by staining the cells with those antisera that were preabsorbed with antigen (10 μg/ml).

Preparation of conditioned medium

The trout PD was cultured in a 24-well plate at a density of 8 PD/well with 1.6 ml serum-free medium (MEM with Earle's salts supplemented with kanamycin; osmotic pressure was 280–290 mosmol) at 15°C under an atmosphere of 95% O_2/5% CO_2. The conditioned medium was collected after 3 days, and was used in the experiment immediately. Control medium was incubated in a well without the PD under the same conditions. Concentrations of released PRL and GH into the medium were 0.2 μg/ml and 100 μg/ml during the incubation, respectively.

Immunoprecipitation

In the trout, a method employing pulse-labeling followed by a specific immunoprecipitation was developed to measure PRL release into the conditioned medium and medium containing added PRL. The cells attached on 96-well plate were preincubated in MEM containing ^{35}35S-methionine (Amersham; 740 kBq/200 μl) for 2 days at 15°C under an atmosphere of 95% O_2/5% CO_2. After preincubation, the culture medium was replaced with serum-free medium (control), the medium containing chum salmon PRL, or the conditioned medium. The cells were then incubated under the same conditions for 2 more days.

At the end of the incubation, the medium was collected and divided into two aliquots. The first was diluted 1 : 5 with 10 mM PBS (pH 7.4) containing 0.1% Triton X-100 and 1% BSA. Fifty μl of the diluted medium were mixed with 50 μl of 1 : 1000 dilution of anti-salmon PRL rabbit serum (LMH), and were incubated at 4°C overnight. The antiserum was sufficient to bind all PRL present in the medium. The second aliquot was similarly incubated with normal rabbit serum. The antibody-bound PRL was precipitated by addition of 100 μl goat anti-rabbit γ-globulin containing 10% polyethylene glycol. After incubation at room temperature for 2 hr, 300 μl 10 mM PBS (pH 7.4) containing 0.1% Triton-X 100 and 1% BSA were added, and the mixture was vortexed and then centrifuged at 2000×g for 60 min. The supernatant was aspirated, and the precipitate was dissolved in 350 μl 0.01 N NaOH. A 300-μl sample was removed from the solution, and mixed with 3 ml scintillation fluid (Aquasol-2, NEN, Boston); the radioactivity was measured in a liquid scintillation counter (Tri-Carb 300, Packard, Downers Grove). The relative amount of [^{35}S]PRL was expressed as the difference between antiserum- and normal serum-precipitated radioactivity.

The specificity of immunoprecipitation was checked using a competition test in which 2.5 and 25 μg salmon PRL or GH were added to the tubes. To determine the percentage of total PRL bound by the antiserum, a trace amount of [^{125}I]salmon PRL was used to monitor recovery. Iodinated-salmon PRL was added to the samples, which were treated in a manner similar to that described above except that the [^{125}I]radioactivity in the precipitates was measured using a gamma counter (Crystal 5424, Packard). The recovery of added PRL from the medium under these conditions was 95%.

Data analysis

The significance of the differences between the means was analyzed by Kruskal-Wallis test for multiple groups followed by Mann-Whitney U-test with the aid of a computer (NEC-PC9801) using programs made by Ishii *et al.* [14].

RESULTS

Hormone release from the organ-cultured PD and the dispersed cells

In the trout, PRL release from the dispersed cells was 10-30 times greater than that from the organ-cultured PD during the experiment for 1–7 days: PRL released from the organ-cultured PD on day 7 was about 10% of the total hormone present in both the medium and the tissue, whereas that from the dispersed cells was about 60% (Fig. 1). There was no significant difference in GH release between the dispersed cells and the organ-cultured PD. Almost 90% of the total hormone were released by day 4.

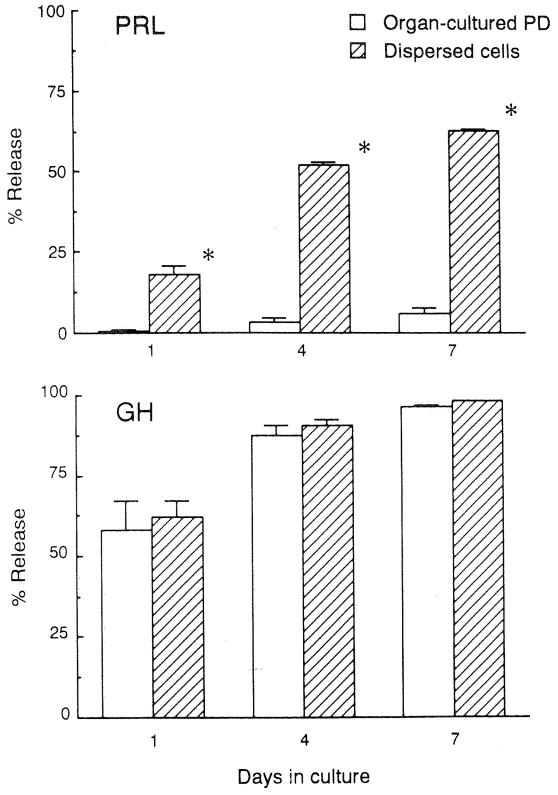

FIG. 1. PRL and GH release from the organ-cultured PD (open column) and from the dispersed cells attached on the plate (hatched column) of the trout pituitary. Data are expressed as means±SEM (n=4). *Significantly different from the corresponding level in the tissue at 5% level.

In the tilapia, release of PRL_{177} from the dispersed cells was greater than that from the organ-cultured PD on day 1. However, there was no difference on days 4 or 7 (Fig. 2). Release of both PRL_{177} and PRL_{188} from the organ-cultured PD and also from the dispersed cells was much greater than

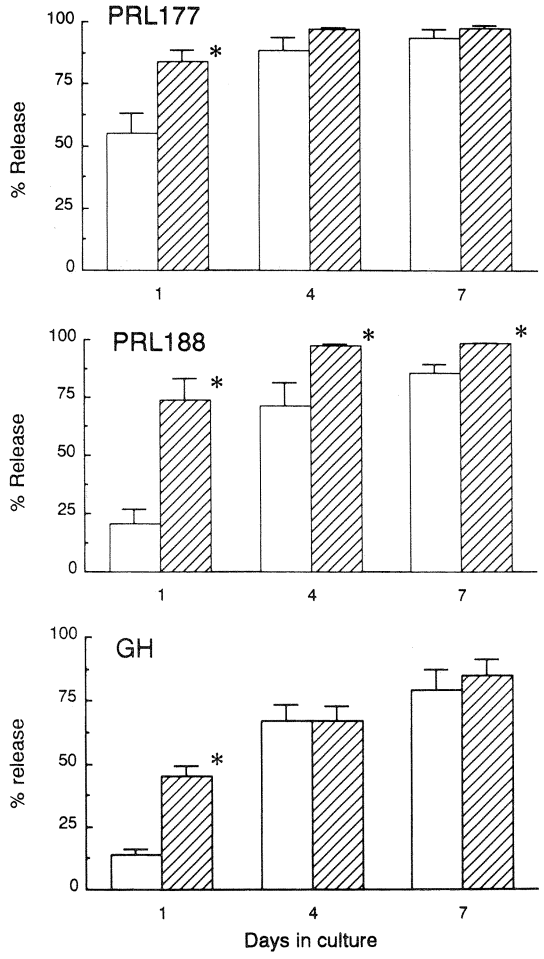

FIG. 2. PRL_{177}, PRL_{188}, and GH release from the organ-cultured PD (open column) and from the dispersed cells attached on the plate (hatched column) of the tilapia pituitary. Data are expressed as means ± SEM (n=4). *Significantly different from the corresponding level in the tissue at 5% level.

the PRL release from the trout pituitary, releasing more than 75% of the total hormone by day 4. PRL_{188} release from the dispersed cells was significantly greater than that from the organ-cultured PD during the experiment. GH release from the dispersed cells was significantly greater than that from the PD tissue on day 1, but, there was no difference on days 4 or 7.

Morphological observation of the cell aggregates

The dispersed cells of both the trout and tilapia, organized into a disk-like structure within one day under the continuous gyratory shaking. Only a small number of single cells remained in suspension. The size of the aggregates was about 1 mm in diameter, 0.2–0.3 mm in height. Figure 3 shows a cell aggregate of the trout pituitary on day 4.

Figure 4 shows light microscopic immunocytochemistry of the aggregates on day 4. The cells appeared to be attached to one another. No nerve endings were observed in the aggregates. Both in the trout and tilapia, most of PRL-immunoreactive (ir) cells were attached to one another as clusters (Figs. 4A and 4C), whereas GH-ir cells appeared to be scattered throughout the aggregates (Figs. 4B and 4C).

Hormone release from cell aggregates and dispersed cells

In the trout, PRL release from the aggregates was significantly lower than that from the dispersed cells except for day 1 of the culture (Fig. 5). There was no difference in GH release between the aggregates and the dispersed cells, releasing more than 90% of the total hormone by day 4.

For the tilapia, there was no difference in PRL_{177} and PRL_{188} release between the aggregate and the dispersed cells (Fig. 6). Percentages of the two PRLs release in the tilapia were again greater than that in the trout. Although GH release from the dispersed cells was significantly greater than that from the aggregates on day 1, there was no difference in the release on days 4 or 7.

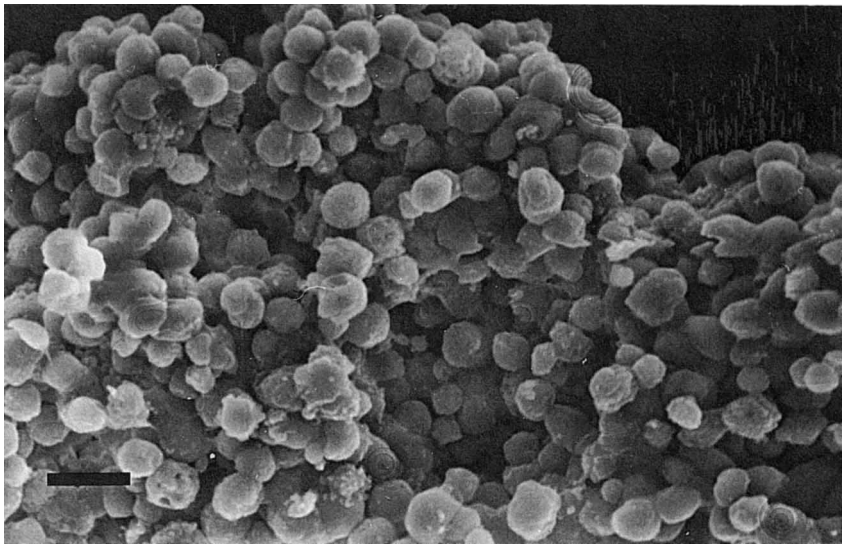

FIG. 3. Scanning electron microscopic photograph of a cell aggregate of the trout pituitary on day 4 of the culture. Scale bar, 10 μm.

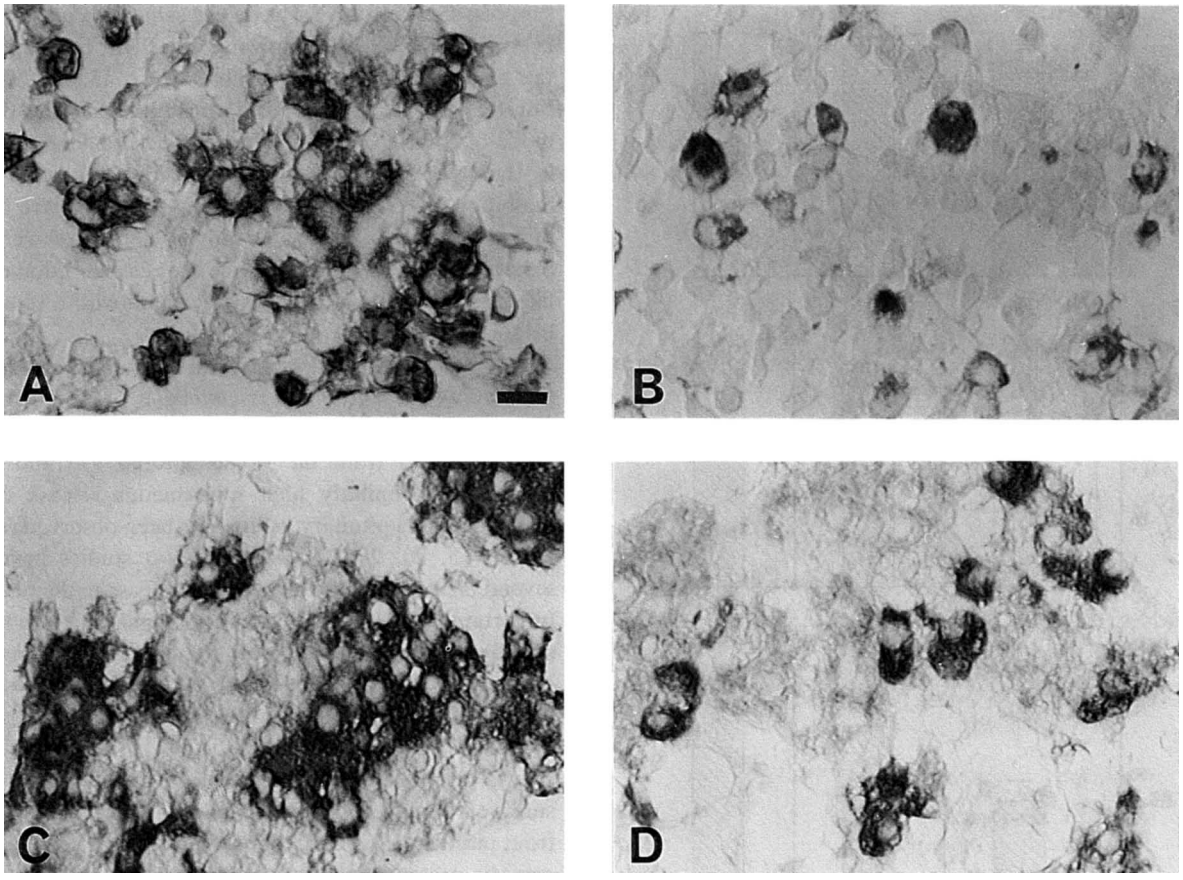

FIG. 4. Light microscopic photographs of cell aggregates of pituitary on 4 day of the culture. A, PRL-immunoreactive (ir) cells in the trout. B, GH-ir cells in the trout. C, PRL-ir cells in the tilapia. D, GH-ir cells in the tilapia. Scale bar, 10 μm.

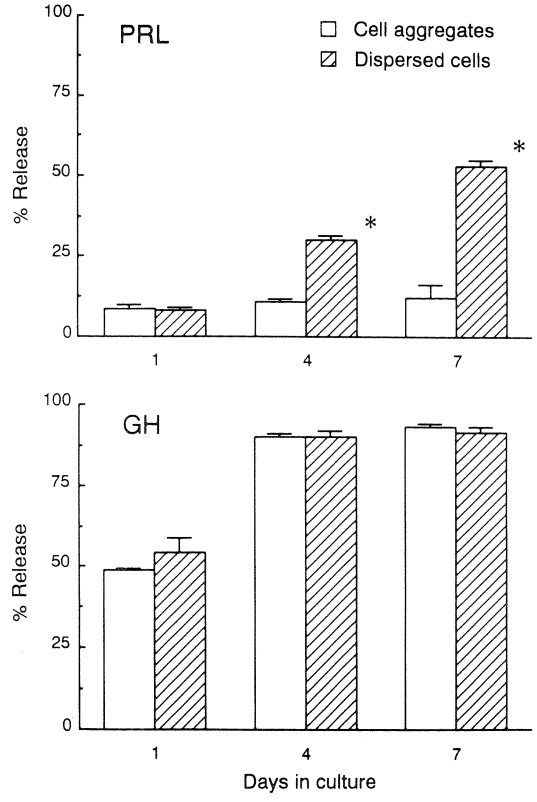

FIG. 5. PRL and GH release from the cell aggregates (open column) and from the dispersed cells attached on the plate (hatched column) of the trout pituitary. Data are expressed as means ± SEM (n=4). *Significantly different from the corresponding level in the aggregate at 5% level.

Effects of salmon PRL and the conditioned medium on PRL release in the trout

As shown in Figure 7, release of newly synthesized PRL from dispersed trout pituitary cells was measured by counting [^{35}S]PRL precipitated by specific antiserum. The addition of salmon PRL to the medium did not affect the PRL release. By contrast, the release of PRL from dispersed cells was inhibited in the medium conditioned by culturing the PD.

DISCUSSION

The present study demonstrates that the organ-cultured pituitary (PD) and the cell aggregates of the rainbow trout (*Oncorhynchus mykiss*) released a smaller proportion of available PRL than that did the dispersed pituitary cells attached on the culture plate. On the other hand, there was no difference in % release of GH among organ-cultured PD, cell aggregate and dispersed cells. Thus, it would appear that the formation of the cells in tissue-like structure does not

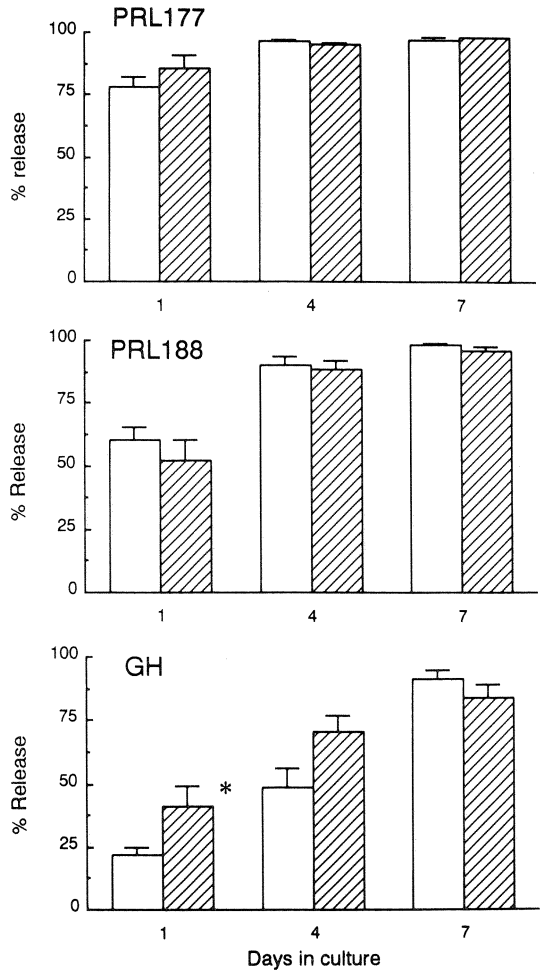

FIG. 6. PRL_{177}, PRL_{188}, and GH release from the cell aggregate (open column) and the dispersed cells attached on the plate (hatched column) of the tilapia pituitary. Data are expressed as means ± SEM (n=4).

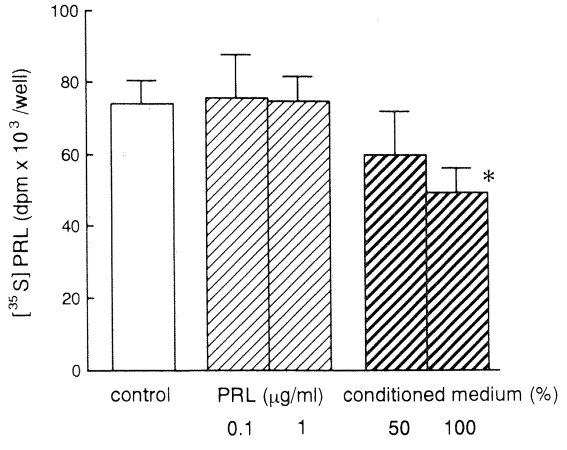

FIG. 7. Effects of salmon PRL and the conditioned medium on [^{35}S]PRL release from the dispersed cells of the trout pituitary attached on the culture plate. Data are expressed as means ± SEM (n=4–5). *Significantly different from the control at 5% level.

suppress GH release. The PD of salmonids and the eel has been found to release relatively little PRL, but large quantities of GH during *in vitro* incubations [1, 15, 28, 29, 35, 36]. For this reason, it has been concluded that the dominant mode of control by hypothalamus over PRL and GH release in the trout is likely to involve stimulation for PRL and inhibition for GH. In the present study, we have observed that dispersion of trout PD cells is associated with an enhancement in PRL release, while the reconstitution of tissue-like structure as aggregates is associated with a reduction in PRL release.

A clearly different picture emerged from our studies of the tilapia (*Oreochromis mossambicus*). Substantial quantities of both PRLs were released from not only the dispersed cells, but also from the organ-cultured PD and the cell aggregates. Similarly high spontaneous release of PRLs from the tilapia pituitary *in vitro* has been observed repeatedly [10, 15, 20, 25, 26, 34]. *In vitro* studies have shown several factors which influence PRL release in the tilapia [22]. Importantly, PRL release from the tilapia pituitary responds directly to small physiological changes in medium osmotic pressure [9, 22]. The salmonid pituitary seems to be quite different in this respect with PRL release appearing independent of osmotic pressure [8, 15, 29, 35]. Quite large and clearly unphysiological reductions in medium osmotic pressure were required to evoke a change in PRL release from the trout pituitary [8]. In the present investigation, the medium osmotic pressure was adjusted to the plasma levels in the trout and tilapia. For this reason, that fact that higher levels of PRL release were observed with the tilapia tissues than with those from the trout is unlikely to be tied to medium osmotic pressure, especially since the medium osmotic pressure used for the tilapia incubations was higher than that used in the trout studies.

The rotation-mediated aggregation of the pituitary cells has been developed to examine cellular organization and functional interactions among cell types [19]. Specific polarization of PRL cells was not observed in aggregates of rat pituitary cells [31]. Hattori *et al.* [11] have observed PRL cells in the trout and goldfish (*Carassius auratus*) pituitary cell aggregates in clusters. In this study, clusters of PRL cells were also observed in the pituitary cell aggregates of the tilapia as well as the trout. Follicular structure is a characteristic of PRL cells of salmonids [3, 13]. In the present study, however, the follicular arrangement of trout PRL cells *in situ* was not reconstituted even after 7 days in the culture. Nevertheless, the release of PRL from the trout PRL cell aggregates was similar to that observed from the intact PD and considerably less than that observed with dispersed PRL cells.

The density of the dispersed cells to form the cell aggregates (5×10^5 cells/ml) was higher than that of the dispersed cells attached on the plate (2×10^4 cells/200 μl). High density of the cultured cells inhibited GH release in carp and gonadotropin release in trout [17, 33]. In the present study, the lower release of tilapia GH from the cell aggregates

than that from the dispersed cells attached on the plate on day 1 might be due to the high density of the cells before the reconstitution of tissue-like structure. However, the difference was disappeared on days 4 and 7. On the other hand, the release of trout PRL from the aggregates was constantly lower than that from the dispersed cells from day 1 to 7. The fact that little amount of PRL was released from the aggregates even on days 4 and 7 suggests that PRL release in trout was inhibited after reconstitution of tissue-like structure in the cell aggregates

Numerous hypothalamic principles have been known to influence on PRL release in the trout *in vitro* [22]. In teleosts, the equivalent of the median eminence is incorporated into the rostral neurohypophysis. In the rainbow trout, aminergic and peptidergic fibers have been shown to directly innervate the PD [22, 30]. In our previous study using organ-cultured trout pituitary, nerve endings were found intact after 8 days in culture [36]. By contrast, no nerve ending was observed in the cell aggregates in this study. Thus, it is unlikely that the suppression of PRL release in the tissue-like structure was caused by hypothalamic hormones.

Prolactin exerts an autoregulatory role on its own release at the pituitary level in the rat [16]. We examined whether a similar possibility might exist in the trout pituitary by adding chum salmon PRL to the dispersed cells with activated PRL release. The amino acid sequence of chum salmon PRL is very close to that of rainbow trout PRL with a difference of only one residue at position 186 in the sequence [18,38]. We added concentrations of salmon PRL (0.1 and 1 μg/ml) that were close to those which we had observed to be released into the medium after 7 days (0.5 μg/ml). Pulse-labeling followed by immunoprecipitation revealed that the addition of salmon PRL had no effect on subsequent PRL release from the dispersed cells. This suggests that the suppression of PRL release in the tissue culture is not likely to involve autoregulation of PRL release.

Porter *et al.* [24] reported an inhibition of PRL release in the rat with transplanted pituitaries containing potential secretors of GH. In investigating this possibility in the trout, we found that the aggregate released 12% of available PRL for 7 days in medium containing 25 μg GH/ml. On the other hand, the dispersed cells of the trout PD released 60% of total PRL for 7 days in the medium containing 44 μg GH/ml (data not shown). These observations show that dispersed cells released substantial quantities of PRL even in the medium containing high concentration of GH. Thus, GH does not seem to be an inhibitory factor for PRL release in the trout.

The fact that PRL release from dispersed cells was suppressed in the medium conditioned by the preincubation of PD tissue suggests that inhibitory factor(s) secreted from the cells in the PD influenced on PRL release in the trout. It is possible that factor(s) released from PRL cells or other cell types in the PD influenced PRL release. The other possibility is that hypothalamic hormone(s) might be leaked from nerve endings remaining in the PD and suppressed PRL release from the dispersed cells. Although not an exact parallel to the situation in the trout, paracrine control of PRL release has been demonstrated in the rat. Prolactin release from the dispersed cells of rat pituitary was stimulated when the incubation medium was conditioned by exposure to gonadotroph-enriched cell culture [6]. Medium conditioned by the posterior pituitary also stimulates PRL release. An involvement of folliculo-stellate cells in PRL release is also suggested in the rat [7, 32]. Further studies will be required not only to identify the factor that regulates PRL release in the relation to the structural arrangement of the cells in the trout but also to assess the possible importance of contact between PRL cells and other specific cell types in the PD tissue. It will also be interesting to clarify whether the difference in the arrangement of PRL cells in the pituitary of lower and higher teleosts provides a basis for the differences in the response of the PRL cells of fishes.

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