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A Two-Receptor Model for Salmon Gonadotropins (GTH I and GTH II)[1]

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ABSTRACT

The possible existence of distinct receptors for salmon gonadotropins (GTH I and GTH II) and the distribution of the receptor(s) were studied through examination of the binding of coho salmon (*Oncorhynchus kistuch*) GTH I and GTH II to membranes from thecal layers and granulosa cells of salmon ovaries. Purified coho salmon gonadotropins were iodinated by the lactoperoxidase method. Crude membrane preparations were obtained from thecal layers, granulosa cells, and whole ovaries of coho salmon in the postvitellogenic/preovulatory phase. Binding of ^{125}I-GTH I to membranes from thecal layers, granulosa cells, and whole ovaries, and binding of ^{125}I-GTH II to thecal layer cell membranes could be inhibited by both GTHs, but GTH I was more potent than GTH II. In contrast, GTH II was more potent than GTH I in inhibiting ^{125}I-GTH II binding to membranes from granulosa cells and whole ovaries, but the inhibition curves were not parallel. Scatchard plot analysis suggested that there was a single type of receptor in the thecal layers for both GTHs, whereas in the granulosa cells there was more than one type of receptor for both GTHs. Based on these results, a two-receptor model for the postvitellogenic/preovulatory salmon ovary is proposed with the following features: 1) there are two types of gonadotropin receptors in the salmon ovary, type I and type II; 2) the type I receptor binds both GTHs, but with higher affinity for GTH I, whereas the type II receptor is highly specific for GTH II and may have only limited interaction with GTH I; and 3) the type I receptor is present in both thecal cells and granulosa cells, whereas the type II receptor is present in granulosa cells.

INTRODUCTION

In mammals and birds, there are separate gonadal receptors for FSH and LH. In mammals, gonadal receptors for FSH and LH are distinct [1–3] and highly specific [4–6]. In addition, CG is generally considered to act through LH receptors [7–9]. In birds, FSH and LH are generally considered to have different gonadal receptors [10–14]. However, it has been shown in the turkey that binding of ^{125}I-FSH to gonadal receptors can be substantially inhibited by turkey LH, whereas turkey FSH was much less potent than LH in inhibiting ^{125}I-LH binding [12]. Highly specific LH receptors were also found in the quail testis using chicken LH and FSH to inhibit ^{125}I-LH binding [14]. These data suggest that the avian FSH receptor is not as specific as the avian LH receptor.

In reptiles and amphibians, the available data are not as clear regarding the existence of separate receptors for FSH and LH. The heterologous binding systems that have been used in many studies make interpretation of results even more difficult. However, the few studies that have used homologous binding assays show that gonadotropin receptors in lower vertebrates are not as specific as those in mammals. In reptiles, sea turtle (*Chelonia mydas*) LH has been shown to inhibit the binding of ^{125}I-FSH to gonadal receptors of several reptilian species, including the sea turtle [15]. In amphibians, one study [16] using homologous FSH and LH of bullfrog (*Rana catesbeiana*) suggested that there might be two types of testicular receptors: one type bound both FSH and LH, and the other type bound primarily LH. However, subsequent reports from that same laboratory showed that bullfrog FSH and LH completely inhibited each other's binding to both hepatic and testicular receptors [17, 18]. As a result, the authors [18] proposed a mechanism by which different biological activities of FSH and LH might be achieved through only a single type of receptor and concluded that there was only one type of gonadotropin receptor in the bullfrog. These results support the view that in the lower vertebrates, FSH and LH share at least some binding sites or receptors; but the apparent differences in the biological activities of FSH and LH [19–22] can also be conveniently explained by the existence of different receptors for the two gonadotropins. Although the proposed mechanism for the bullfrog gonadotropin receptor [18] may be functionally adequate, the possibility that there are different receptors for FSH and LH in the lower vertebrates cannot be discounted.

In fishes, it was thought until recently that only a single gonadotropin existed; therefore the possibility of different types of receptors was not envisioned. The purification of two chemically different salmon gonadotropins, GTH I and GTH II [23, 24], has raised the question whether there are multiple gonadal receptors for these hormones. Recently we reported the presence of specific gonadal receptors for coho salmon GTH I and GTH II, and optimized in vitro conditions for hormone-receptor interactions [25]. In that

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study, it was shown that GTH I was more potent than GTH II in inhibiting the binding of both ^{125}I-GTH I and ^{125}I-GTH II to membranes from coho salmon postovulatory ovaries. However, GTH I was 7–8 times more potent than GTH II in inhibiting ^{125}I-GTH I binding, whereas GTH I was only 2–3 times more potent than GTH II in inhibiting ^{125}I-GTH II binding. On the basis of these results we suggested that there might be some shared and some separate binding sites. However, the evidence was insufficient to permit definitive conclusions about specific receptor types.

Because we were unable to demonstrate clearly the existence of multiple receptor types using membranes from the postovulatory ovaries, we decided to use membranes from isolated thecal layers and granulosa cells for binding studies. In this paper, we report evidence suggesting the presence of at least two types of receptors for salmon gonadotropins. In addition, we propose a two-receptor model for tissue distribution of the two types of receptors in postvitellogenic/preovulatory ovarian follicles of salmonids.

MATERIALS AND METHODS

Hormones, Iodination and Binding Assay Procedures

Coho salmon GTH I and GTH II were purified by Swanson et al. [24]. Unless otherwise indicated, all chemicals used in the experiments were purchased from Sigma Chemical Co., St. Louis, MO. Methods for radioiodination of GTHs and the optimization of binding conditions have been described previously [25]. Nonspecific binding was estimated in the presence of 1 μg/tube (4 μg/ml) SG-G100 (a partially purified glycoprotein extract of mature coho salmon pituitaries containing GTH I, GTH II, and trace quantities of thyroid-stimulating hormone) prepared by Swanson et al. [24]. The only modification of the binding assay procedure was the repurification of radioiodinated GTHs as follows. After desalting of the radioiodinated GTHs, the peak of labeled hormone was subjected to gel filtration chromatography using a column of Sephadex G-75 superfine (1 × 50 cm; Pharmacia-LKB Biotechnology, Inc., Piscataway, NJ). The column was equilibrated with 20 mM Tris buffer containing 0.5% BSA (RIA grade) and 0.05% NaN_3, and run at a flow rate of 8 ml/h. Fractions of 0.5 ml were collected. The repurification of iodinated hormone improved specific binding of both gonadotropins (about 10%), possibly due to the elimination of aggregates, dissociated subunits, and some free Na^{125}I. Therefore, for the experiments described in this study, only repurified labeled hormones were used. The specific activity of the labeled hormones ranged from 20 to 50 μCi/μg as estimated by the self-displacement method [26].

Tissue Sources, Separation, and Membrane Preparation

Postvitellogenic/preovulatory ovaries of coho salmon were collected at Domsea Farms Inc. (Rochester, WA). The reason for choosing ovaries of this stage was that the ovarian follicles were at their maximal size; this greatly facilitated the separation of the follicular cell layers. Tissues were removed from fish and frozen on dry ice immediately after the fish were killed. The samples were stored at −76°C until membrane preparations were made. Unless otherwise indicated, the samples were placed on ice, and the buffer solutions were chilled on ice at all times during the experiments. Frozen tissues were thawed in modified Hanks' balanced salt solution (NaCl, 106.9 mM; KCl, 5.4 mM; $MgCl_2$, 20 mM; $MgSO_4$, 0.8 mM; KH_2PO_4, 0.5 mM; Na_2HPO_4, 0.3 mM; $NaHCO_3$, 8 mM; HEPES, 10 mM; pH 7.5). The tissues were placed in a shallow glass dish containing modified Hanks' solution; the thecal layers and chorion-granulosa layers (layers in which the granulosa cells were attached to the chorion or zona radiata) were physically separated with fine-tipped watchmaker's forceps as previously reported [27]. Egg yolk materials in the separated thecal and chorion-granulosa layers were washed away using modified Hanks' solution, followed by filtering the solution through a 500-μm nylon mesh. This process detached some granulosa cells from the chorion; therefore, the filtrate was saved and centrifuged at 2000 × *g* for 5 min at 2°C to collect the granulosa cells. The remaining granulosa cells on the chorion were detached using a reported procedure [28] with slight modification. The chorion-granulosa layers were stirred for 2 h in 20 mM Tris-HCl containing 0.6% NaCl, pH 7.5, with a magnetic stirrer in a beaker on ice. The solution was filtered through the 500-μm nylon mesh, and the filtrate was centrifuged (as above) to collect the detached granulosa cells. The chorion was homogenized and saved (the membrane preparation procedure could not be applied to the chorion, because the broken chorion would sediment at 600 × *g*). The pellets of granulosa cells and the thecal layers were then washed three times (first with modified Hanks' and then twice with 20 mM Tris-HCl, pH 7.5, 10–15 ml buffer per gram tissue) followed by centrifugation (as above) after each wash. For the whole ovaries, the frozen tissue was minced with a razor blade in a petri dish. The minced tissue was washed three times with modified Hanks' solution followed by two washes with Tris-HCl to remove the yolk materials. Following each wash the minced tissue was recovered by centrifugation as above. The pellets of thecal layers, granulosa cells, and minced ovaries were then homogenized in Tris-HCl, and membrane preparations were made as described previously [25]. Protein concentrations of the preparations were measured by a modified Lowry method [29].

Data Calculation and Plotting

Parallelism of the inhibition curves was evaluated with small-sample *t*-test after logarithmic transformation of the hormone concentrations [30]. The inhibition curves and Scatchard plots were done with the Cricket Graph (CA Associates, Malvern, PA) and/or the Statview 512+ (Brain

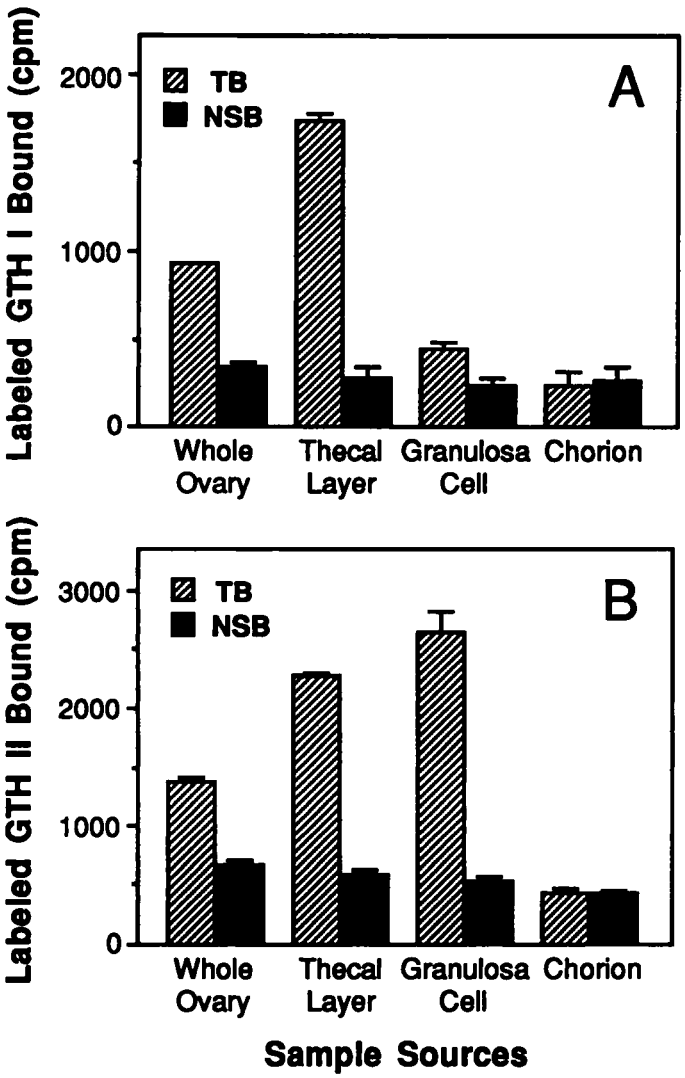

FIG. 1. Total binding (TB) and nonspecific binding (NSB) of ^{125}I-GTH I (A) and ^{125}I-GTH II (B) to membrane preparations from different ovarian tissues and chorion homogenate of coho salmon. For each measurement, 400 μg protein of each preparation and 20 000 cpm of labeled hormone were used. Each data point was plotted as the mean and range of triplicate measurements except where the range lies within the symbol.

Power, Inc., Calabsas, CA) programs on Macintosh computers. The dissociation constants (K_d) of hormone-receptor interactions in the granulosa cell and whole-ovary membrane preparations were estimated by least-squares best fit of two linear regression lines to all the data points using an IBM PC program, and the receptor capacities were calculated according to the method of Klotz and Hunston for two independent sites [31].

RESULTS

Specific binding (total minus nonspecific binding) of ^{125}I-GTH I and ^{125}I-GTH II was observed in membranes from whole ovaries, thecal layers, and granulosa cells, but not in the chorion homogenate (Fig. 1). The ratio of specific binding to total binding was slightly greater for GTH I than for GTH II in the membranes from whole ovaries (63.7% for GTH I, 51.9% for GTH II) and thecal layers (82.1% for GTH I, 73.9% for GTH II). In contrast, in the granulosa cell membranes, the ratio for GTH II (80.2%) was almost twice that for GTH I (41.8%). Therefore, it was reasoned that either there were different receptors or there was a different distribution pattern of the receptors in the thecal layers and the granulosa cells.

Next, the relative abilities of GTH I and GTH II to inhibit the binding of radioiodinated GTHs to cell membranes from isolated thecal layers and granulosa cells were examined. In the thecal layer cell membranes, the binding of ^{125}I-GTH I or ^{125}I-GTH II was inhibited substantially by both GTHs, but GTH I was more potent than GTH II at almost all concentrations tested (Fig. 2). The test for parallelism between

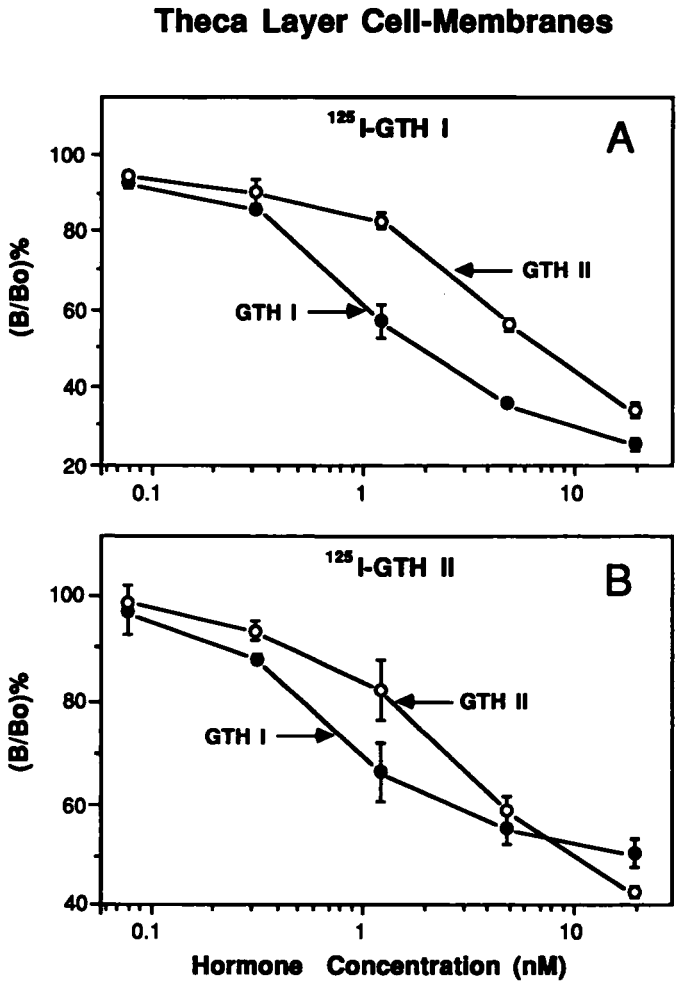

FIG. 2. Inhibition curves for the binding of ^{125}I-GTH I (A) and ^{125}I-GTH II (B) to membrane preparations from isolated thecal layer in the presence of different concentrations of GTH I and GTH II. Nonspecific binding was 22.8% of total binding for GTH I and 36.6% for GTH II. B = binding in the presence of different concentrations of unlabeled hormone; Bo = binding in the absence of unlabeled hormone, i.e., total binding. Each data point was plotted as the mean and range of duplicate measurements except where the range lies within the symbol.

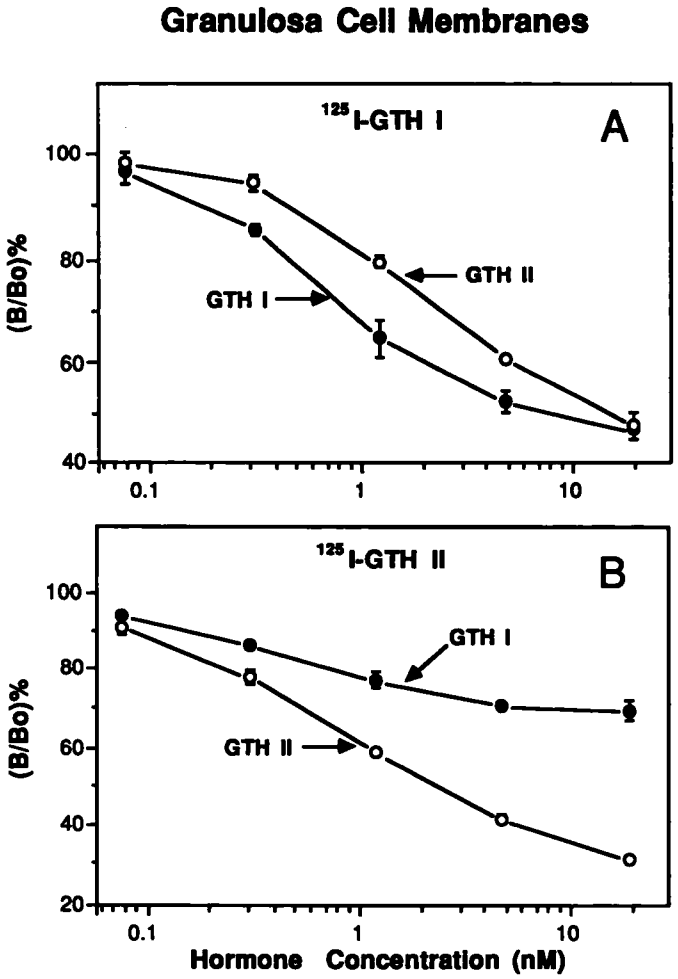

FIG. 3. Inhibition curves for the binding of ^{125}I-GTH I (A) and ^{125}I-GTH II (B) to membrane preparations from isolated granulosa cell in the presence of different concentrations of GTH I and GTH II. Nonspecific binding was 41.7% of total binding for GTH I and 23.9% for GTH II. B = binding in the presence of different concentrations of unlabeled hormone; Bo = binding in the absence of unlabeled hormone, i.e., total binding. Each data point was plotted as the mean and range of duplicate measurements except where the range lies within the symbol.

the inhibition curves of ^{125}I-GTH I and ^{125}I-GTH II by GTH I and GTH II revealed no difference ($0.55 < p < 0.65$). Therefore, it was reasoned that in the thecal layers the two GTHs might be interacting with the same type of receptor, one that had a higher affinity for GTH I. This type of receptor was designated as the type I receptor. In the granulosa cell membranes, binding of ^{125}I-GTH I was also inhibited by both GTHs, and GTH I was more potent than GTH II (Fig. 3A). The test for parallelism between the two curves in Figure 3A revealed no difference ($0.50 < p < 0.55$). Therefore, it was reasoned that the binding might be due to the same type I receptor as in the thecal layer cell membranes. In contrast, the inhibition curves of ^{125}I-GTH II binding to the granulosa cell membranes by the two GTHs were divergent (Fig. 3B), and GTH II was more potent than GTH I at all concentrations tested. The test for parallelism of the inhibition curves in Figure 3B revealed a significant difference ($0.01 < p < 0.05$). These data suggested that GTH II was interacting with at least two types of receptors in the granulosa cell membranes: GTH II binding to one type of receptor (type I) could be inhibited by GTH I, but its binding to the second type of receptor could not be substantially inhibited by GTH I. This second type of receptor was designated as the type II receptor.

Finally, the relative abilities of GTH I and GTH II to inhibit the binding of radioiodinated GTHs to the whole-ovary cell membranes were examined (Fig. 4). Because the whole-ovary cell membranes were a mixture of the thecal layer cell membranes and the granulosa cell membranes, both the type I and the type II receptors should be present. As expected, the binding inhibition curves for ^{125}I-GTH I to the whole-ovary cell membranes resembled that of the thecal layer and granulosa cell membranes (Figs. 2A and 3A). The test for parallelism between the two curves in Figure

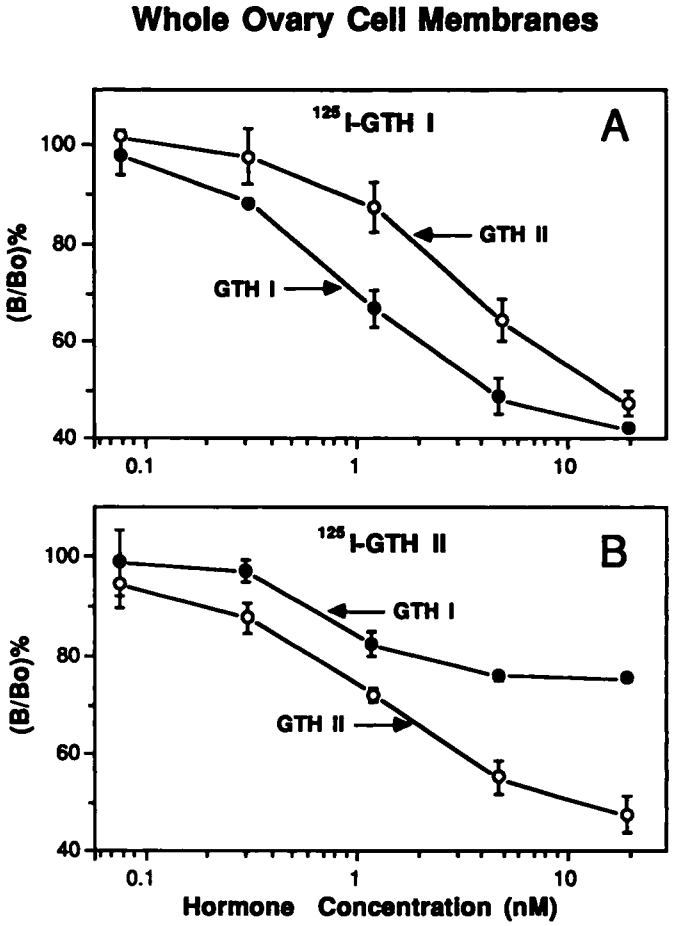

FIG. 4. Inhibition curves for the binding of ^{125}I-GTH I (A) and ^{125}I-GTH II (B) to membrane preparations from whole ovary in the presence of different concentrations of GTH I and GTH II. Nonspecific binding was 36.7% of total binding for GTH I and 38.9% for GTH II. B = binding in the presence of different concentrations of unlabeled hormone; Bo = binding in the absence of unlabeled hormone, i.e., total binding. Each data point was plotted as the mean and range of duplicate measurements except where the range lies within the symbol.

4A revealed no difference ($0.50 < p < 0.55$). In addition, the binding of ^{125}I-GTH II to the whole-ovary cell membranes (Fig. 4B) resembled that of the granulosa cell membranes (Fig. 3B). The two inhibition curves (Fig. 4B) appeared nonparallel, although the difference in their slopes was not statistically significant ($0.1 < p < 0.15$). Therefore, the data suggested that the whole-ovary cell membranes also contained both the type I and the type II receptors.

Using the data from the inhibition curves, Scatchard analysis was performed to further resolve specific receptor types and estimate binding affinities and capacities (Fig. 5; Table 1). Scatchard plots for the binding of both GTH I and GTH II to thecal layer cell membranes were linear (Figs. 5, A and B), indicating the presence of only a single type of receptor. This is consistent with the notion that there is only one type of receptor in the thecal layer. Also in good agreement with the inhibition curves (Figs. 3 and 4), Scatchard plots for the binding of both GTH I and GTH II to granulosa cell (Figs. 5, C and D) and whole-ovary membrane preparations (Figs. 5, E and F) were curvilinear, suggesting the existence of more than one class of receptors. Since competitive binding experiments (Figs. 2–4) suggested that the type I receptor had a higher affinity for GTH I than for GTH II, it was reasoned that the high affinity binding sites for GTH I and the low affinity binding sites for GTH II in the granulosa cell and whole-ovary membranes were the type I receptors. The competitive binding experiments (Figs. 3 and 4) also showed that the type II receptor had greater specificity for GTH II. Therefore, it was reasoned that the high affinity binding sites for GTH II and the low affinity binding sites for GTH I in the granulosa cell and whole-ovary membrane preparations were the type II receptors. The estimated affinities (expressed as K_d) of hormone-receptor interactions and the tissue receptor capacities (expressed as fmol/mg protein of the membrane preparations) are shown in Table 1. It should be pointed out, however, that these estimates are based on limited data; therefore additional experiments are needed for more precise estimation of affinities and capacities.

DISCUSSION

The results of the present study indicate that GTH I and GTH II showed different binding characteristics to membrane receptors from separated thecal layers and granulosa cells of the postvitellogenic/preovulatory salmon ovary. In the thecal layer membranes, the binding of ^{125}I-GTH I and ^{125}I-GTH II was inhibited by both GTH I and GTH II, but GTH I was more potent. In the granulosa cell membranes also, the binding of ^{125}I-GTH I was inhibited by both GTHs and GTH I was more potent; however, the binding of ^{125}I-GTH II was substantially inhibited by GTH II but only slightly inhibited by GTH I, and the inhibition curves by GTH I and GTH II were not parallel. In addition, Scatchard plot analysis indicated that there was only a single class of receptor in the thecal layers, whereas in the granulosa cells there were at least two classes of receptors. On the basis of these results, we propose a two-receptor model with the following features: 1) there are at least two types (or classes) of gonadotropin receptors in the postvitellogenic/preovulatory ovary of coho salmon, which we have designated type I and type II; 2) the type I receptor binds both GTH I and GTH II but with higher affinity for GTH I, whereas the type II receptor is highly specific for GTH II and may have only limited interaction with GTH I; and 3) the type I receptor is present in both the thecal and granulosa cells, whereas the type II receptor is present in the granulosa cells. A graphic illustration of this model is shown in Figure 6.

To our knowledge, this is the first time that more than one type of gonadotropin receptor has been demonstrated in any species of fish. The significance of this model is partly revealed by the established steroidogenic activities of thecal and granulosa cells [32] and the steroidogenic activities of the two GTHs [33]. According to the two-cell model for ovarian steroid production in salmon [32] during vitellogenesis, thecal cells produce testosterone, which is aromatized to estradiol in granulosa cells. Vitellogenin production by the liver is dependent on the production of estradiol. After completion of vitellogenesis during final maturation and ovulation, thecal cells produce 17α-hydroxyprogesterone, which is converted to 17α, 20β-dihydroxy-4-pregnen-3-one (DHP) in granulosa cells. Resumption of meiosis and ovulation are dependent on the production of DHP. It has been shown that blood levels of GTH I are elevated during vitellogenesis and decline during final maturation, whereas the elevation of blood GTH II levels is coincident with final maturation/ovulation [34–36]. Bioassays for steroidogenic activities have shown that both thecal and granulosa layers of salmon ovarian follicles are responsive to GTH I and GTH II [33], but there are differences in potency. The ability of GTH II to interact with both types of receptors in granulosa cells, as suggested by the proposed model, may in part explain why GTH II is more potent than GTH I in stimulating DHP production by granulosa cells [33]. In salmon during final maturation and ovulation, the increase in blood levels of GTH II may act through the type I receptor in thecal cells to stimulate 17α-hydroxyprogesterone production, and through both type I and type II receptors or through only type II receptors in granulosa cells to stimulate DHP production.

Although the proposed model is a logical interpretation of the results, more complicated schemes are possible. Furthermore, discrepancies in the data may suggest alternative interpretations. For example, the estimated numbers of receptors in the thecal layer cell membranes for GTH I and GTH II theoretically should be the same if GTH I and GTH II are binding to the same receptors; but estimation by Scatchard plot suggests slightly more receptors for GTH II than for GTH I (Fig. 5A; Table 1). This may be due to contamination of the thecal layers by the granulosa cells. It has

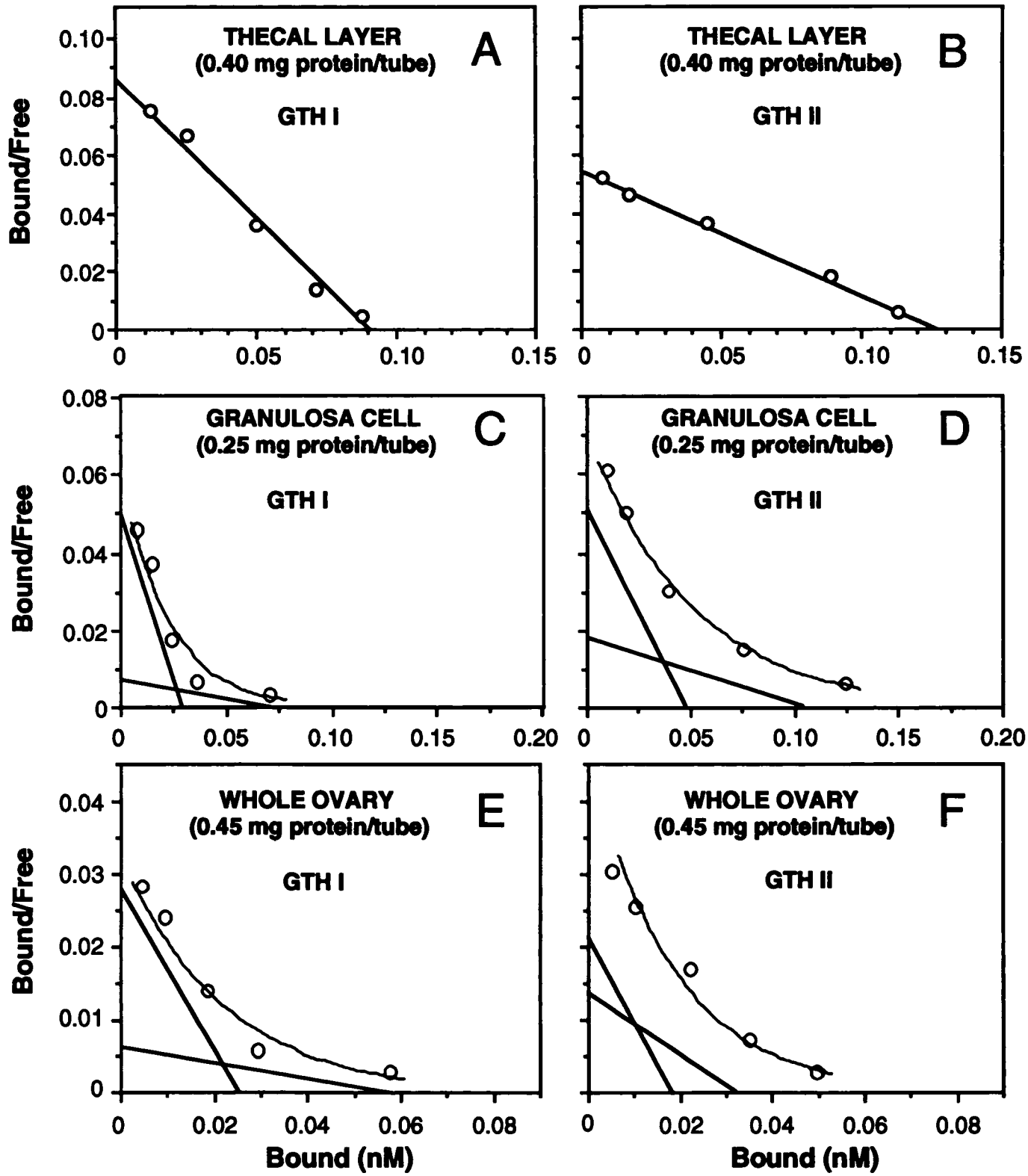

FIG. 5. Scatchard plots for GTH I and GTH II binding to membrane preparations from isolated thecal layer (A, B), granulosa layer (C, D), and whole ovary (E, F). Each data point was the mean of duplicate measurements and corrected for nonspecific binding before plotting.

been shown that with the separation technique used, the thecal layer preparations are sometimes contaminated with granulosa cells (less than 10% of the total number of cells present), whereas the granulosa cell preparations are completely free of thecal cells [27]. As shown in Figure 2B, although GTH I was more potent than GTH II in inhibiting binding of labeled GTH II to thecal layer membranes at most concentrations tested, GTH II appeared more potent at the highest concentration (20 nM). These data are consistent with the assumption that the thecal layer preparations may have been contaminated with granulosa cells; but the extent of the contamination was not estimated in our study. Alternatively, a small number of type II receptors may be present in the thecal cells. In addition, major differences exist in the estimated numbers of receptors of both GTH I and GTH II binding to the same type of receptor in different tissues (Table 1). This could partly be due to the limited number of data points at the lower end of the Scatchard plots (at higher concentrations of unlabeled hormones). The small number of data points makes these estimates imprecise; further experimental analyses are required for greater precision.

The estimates of receptor affinities are subject to the same error, particularly for the low affinity sites. However, consistencies in the data were observed (Table 1). The affinity estimates for GTH I binding to the presumed type I receptor in the thecal layer, granulosa cell, and whole-ovary membranes were in the same order of magnitude (K_d: 0.54–1.04 nM) and were consistent with our previous finding [25] for GTH I binding to postovulatory ovarian membranes (K_d = 0.87 nM). The affinity estimates for GTH I binding to the presumed type II receptor in granulosa cell and whole-ovary membranes were almost identical (K_d: 9.28 and 9.58 nM). Similarly, the affinity estimates for GTH II binding to the presumed type II receptor in granulosa cell and whole-ovary membranes were equivalent (K_d: 0.97 and 1.04 nM). However, the K_d for GTH II binding to the presumed type I receptor in the three membrane preparations ranged from 1.89 to 5.62 nM. Our previous estimate for the affinity of GTH II binding to the postovulatory ovarian membrane preparations was 2.76 nM (K_d), which is within the range of our present estimates for GTH II binding to the type I receptor and is comparable to the K_d for GTH II binding to the thecal layer receptor (K_d = 2.34 nM). This may be due to the prevalence of type I receptors in the postovulatory ovarian membranes (discussed below).

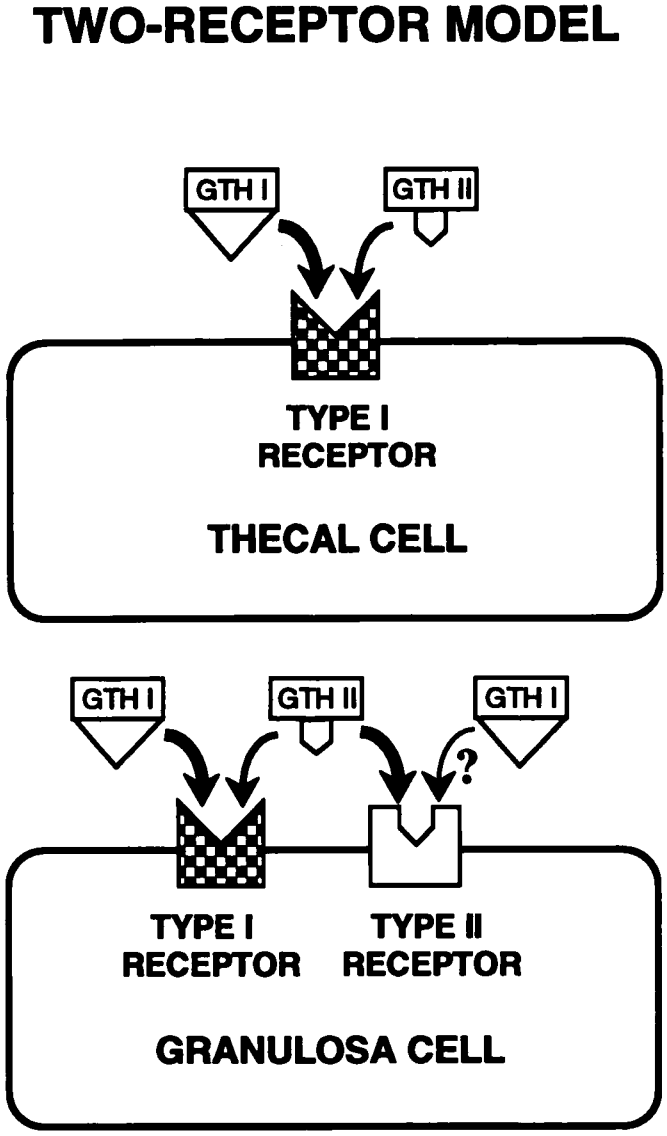

FIG. 6. Graphic illustration of the two-receptor model for salmon gonadotropin binding to postvitellogenic/preovulatory ovaries; see text for details of the model. Heavy arrow lines indicate higher affinity interactions. Thin arrow lines indicate lower affinity interactions.

Because the tissues used in our study were obtained from a few postvitellogenic/preovulatory fish, this two-receptor

TABLE 1. Summary of Scatchard analysis for GTH I and GTH II binding.

			Granulosa cell		Whole ovary	
	Hormone	Thecal layer	High affinity	Low affinity	High affinity	Low affinity
Dissociation constant, K_d (nM)	GTH I	1.01[a]	0.54[a]	9.28[b]	0.93[a]	9.58[b]
	GTH II	2.34[a]	0.97[b]	5.62[a]	1.04[b]	1.89[a]
Capacity (fmol/mg protein)	GTH I	55.27[a]	30.30[a]	73.80[b]	14.33[a]	32.94[b]
	GTH II	80.69[a]	49.00[b]	111.30[a]	10.22[b]	18.11[a]

[a]Presumed type I receptor.
[b]Presumed type II receptor.

model may not be applicable to other stages of follicular development. It is likely that the types, the numbers, and/or the distribution of the receptors are regulated at different developmental stages and under different physiological conditions. As we have shown previously [25] in postovulatory ovaries of coho salmon, the binding data failed to demonstrate clearly the existence of two receptor types. It is unclear whether this was due to loss of the type II receptor and/or loss of granulosa cells after ovulation. Histology of postovulatory ovaries of salmon has revealed frequent granulosa cell abnormalities compared to postvitellogenic/preovulatory ovaries (Dr. S. Miwa, National Research Institute of Aquaculture, Mie, Japan, personal communication). It would not be surprising for granulosa cells or their receptors to be damaged by proteolytic enzymes activated during ovulation, especially in a semelparous fish (i.e., a fish whose life history involves only a single reproductive event followed by death).

In a study in amago salmon (*O. rhodurus*), it was shown that the number of gonadotropin receptors per follicle in both thecal and granulosa layers increases during oogenesis [28]. However, because the nature of the salmon gonadotropin preparation (i.e., the relative content of GTH I and GTH II) used in that study is not known, the data do not provide information concerning receptor types. It would be interesting to evaluate changes in the number, tissue distribution, and affinity of the two receptor types during follicular development and in response to hormonal treatment, e.g., steroid hormone and/or gonadotropin administration. Information derived from such experiments should lead to valuable insight concerning the regulation of gonadotropin receptors.

In mammals and birds, follicular tissue distribution of both FSH and LH/CG receptors has been investigated. In mammalian ovaries, FSH receptors are located exclusively in granulosa cells [37–39], whereas LH receptors are in thecal, interstitial, luteal, and mature granulosa cells [9]. Both FSH and LH receptor numbers are subject to regulation by both reproductive hormones (gonadotropins and sex steroids) and nonreproductive hormones (prolactin and growth factors) [39–45]. In avian ovaries, FSH receptors are found in granulosa cells, whereas LH receptors are found in both theca interna (and possibly theca externa) and granulosa cells [46]. Both FSH and LH receptors are regulated during the avian reproductive cycle [47–50]. Although the steroidogenic activities of isolated thecal and granulosa cells have been studied [46], to our knowledge no binding studies with both FSH and LH using membranes from isolated follicular cells have been reported in any avian species. However, it has been shown recently in the Japanese quail that LH receptor numbers decreased abruptly in the theca layer of the largest follicle that was expected to ovulate within 24 h, while LH receptor numbers in the granulosa layer did not show significant changes [51].

Another interesting question is whether the two-receptor model proposed for salmon can be applied to amphibians and reptiles. In many earlier studies heterologous hormones were used. Due to complications in interpretation of results from these studies, only experiments using homologous hormones were considered for the purpose of the present discussion. It has been shown that in amphibians, FSH and LH binding to testicular and hepatic receptors can be inhibited by each other [16–18]. Our data are comparable to the data obtained from FSH and LH binding to bullfrog testicular receptors [16, 18]: 1) the inhibition curves of ^{125}I-FSH by FSH and LH resemble the inhibition curves of ^{125}I-GTH I by GTH I and GTH II; 2) the inhibition curves of ^{125}I-LH by FSH and LH resemble those of ^{125}I-GTH II by GTH I and GTH II in membranes from whole ovaries and granulosa cells. Although the positive cooperative action of FSH on the binding of LH to bullfrog testicular receptors as proposed by Yamanouchi and Ishii [18] cannot be ruled out, it is also possible that different gonadotropin receptors may exist in amphibians as proposed for the salmon. The data from bullfrog [16, 18] could also be explained by the two-receptor model proposed here. Due to the limited availability of highly purified salmon gonadotropins at the time of our study, it was not possible to conduct experiments using extremely high concentrations of gonadotropins to test whether GTH I and GTH II could inhibit each other's binding completely. Therefore, this question remains to be addressed, although the inhibition curves in Figure 3B do suggest that GTH I is unlikely to substantially inhibit GTH II binding even at higher concentrations. In reptiles, e.g., sea turtle, considerable inhibition of ^{125}I-FSH binding to ovarian homogenate by LH has been shown [15], and the inhibition curves of ^{125}I-FSH binding in the presence of FSH and LH also resemble the inhibition curves of ^{125}I-GTH I binding in the presence GTH I and GTH II. It is regrettable that no parallel experiments were conducted with ^{125}I-LH in the sea turtle. It would be interesting in the future to test the existence of multiple receptor types in amphibians and reptiles using membranes from isolated follicular layers.

It has been shown that the amino acid sequence of salmon GTH I β-subunit is most similar to that of bovine FSH, whereas salmon GTH II β-subunit is most similar to that of bovine LH [52]. In addition, the production and secretion of GTH I is associated with gonadal growth, as is that of tetrapod FSH; while the production and secretion of GTH II is associated with ovulation in female salmon, as is that of tetrapod LH [34–36]. Therefore, for the purpose of discussion, if we assume that GTH I is the fish FSH, and that GTH II is the fish LH, then the type I receptor is the FSH receptor, and the type II receptor is the LH receptor. Thus, a general pattern emerges concerning the specificity of gonadotropin receptors in nonmammalian vertebrates: FSH receptors are low in specificity and are capable of considerable interaction with LH, whereas LH receptors are highly

specific. It must be pointed out, however, that this pattern is deduced mostly from in vitro binding studies; therefore, the physiological significance of this interaction awaits future study.

In conclusion, the present study provides evidence suggesting the existence of at least two types of gonadotropin receptors in salmon, which we have designated as type I and type II receptors. The type I receptor binds both GTH I and GTH II, but with higher affinity for GTH I, whereas the type II receptor binds GTH II specifically and may have only limited interaction with GTH I. The type I receptor exists in both thecal layers and granulosa cells, whereas the type II receptor exists in granulosa cells. It is hoped that this concept will open up new areas of research and stimulate interest in gonadotropin receptors in the lower vertebrates. Current subjects of ongoing studies include possible seasonal changes in GTH receptor type and density, characterization of the molecular nature of the two receptors, determination of the number of GTH receptors in the testes, and cellular localization of GTH receptors using autoradiographic techniques.

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