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GENERAL AND COMPARATIVE ENDOCRINOLOGY 78, 110–122 (1990)

# A Rapid and Sensitive ELISA for Rainbow Trout Maturational Gonadotropin (tGtH II): Validation on Biological Samples; *in Vivo* and *in Vitro* Responses to GnRH

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A rapid and sensitive heterologous enzyme-linked immunosorbent assay (ELISA) was developed to measure rainbow trout maturational gonadotropin. Purified salmon maturational gonadotropin (sGtH II) was used as reference hormone. Optimization of the procedure was performed by using an anti-βsGtH serum. Two procedures were developed: an equilibrium assay (which did not involve a preincubation step) which lasted for 8 hr and a nonequilibrium assay (which involved a preincubation step) which lasted for 26 hr. The nonequilibrium assay gave the best sensitivity (70 pg/ml sample). GtH II measurements on *in vivo* and *in vitro* samples from GnRH analogs or sGnRH experiments showed that the ELISA procedure could be used over a wide range of concentrations. The method was validated by comparing GtH II concentrations measured by both RIA and ELISA. © 1990 Academic Press, Inc.

The development of more precise and sensitive techniques has generated very important changes in some of the ideas and strategies in the field of endocrinology. Reliable hormone measurements can now be made on a few microliters of plasma: in this way, the animal physiology is not disturbed by successive samplings which are required, for example, to detect episodic hormone secretion (Atkinson *et al.*, 1970; Dierschke *et al.*, 1970; Knobil, 1980; Garnier *et al.*, 1978; Ortavant *et al.*, 1982; Zohar *et al.*, 1986). Radioimmunoassays (RIAs) for gonadotropins (GtHs) have become progressively more sensitive over the years. In some physiological situations, however, (e.g., juvenile fish, beginning of gametogenesis) and *in vitro* (e.g., whole pituitary perifusions), the GtH levels are especially low and the classical RIA techniques are still not sensitive enough to measure them (Bailhache *et al.*, 1989). Recently, enzyme-linked immunosorbent assays (ELISAs) for hormones have been developed (Farrington and Hymer, 1987; Manquez *et al.*, 1987; Signorella and Hymer, 1984; Spearow and Trost, 1987; Yamamoto and Kato, 1986). Since these assays do not use radioactive molecules, require less time than RIAs, and, in some cases, are more sensitive than RIAs (specially with the use of the peroxidase–anti-peroxidase technique), they prove a good alternative to RIAs.

The aim of our study was to develop a rapid and sensitive ELISA for rainbow trout maturational gonadotropin (tGtH II, according to Suzuki *et al.*, 1988). After optimization of the procedure, tGtH II levels were measured in plasma samples from gonadotropin-releasing hormone analog (GnRHa)-treated female trout and in perifusion

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samples from a trout brain/pituitary preparation perifused with salmon GnRH (sGnRH). A comparison between ELISA and RIA tGtH II measurements was made.

## MATERIALS AND METHODS

Salmon (*Oncorhynchus tschawytscha*) maturational gonadotropin, which has been found to correspond to the sGtH II of Suzuki *et al.* (1988), was prepared according to Breton *et al.* (1978). Purified sGtH II was used as reference gonadotropin for RIA and ELISA procedures. sGtH II subunits were prepared from the same material. Antibodies against the subunits were raised in rabbits and have already been validated for radioimmunoassay (Breton *et al.*, 1984).

### *Animals*

For *in vivo* experiments, the rainbow trout (*Salmo gairdneri*) were obtained from an experimental fish farm. They were 2 years old and from an autumn-spawning strain. The experiment was performed in late October in nonovulated females taken from a population in which 30% of the animals had already spawned. For *in vitro* experiments, rainbow trout were kept in the laboratory in 500-liter aquaria with fresh water at 15° and under a natural photoperiod. These trout were 18 months old and at the early stage of exogenous vitellogenesis (oocyte diameter <0.5 mm).

### *In Vivo GnRH Treatments*

Four groups of six animals received an intramuscular injection under the dorsal fin, with a saline solution (control group); 20 μg/kg body weight of [D-tryptophan$^6$]luteinizing hormone-releasing hormone ([D-Trp$^6$]LH-RH) purchased from Sigma Chemical Co.; 200 μg/kg body weight of a slow-release form microencapsulated [D-Trp$^6$]LH-RH (Triptoreline from Ferring); or microencapsulated [D-arginine$^6$, proline$^9$ N-ethylamide]sGnRH ([D-Arg$^6$,Pro$^9$ NHEt]sGnRH) releasing 20 μg/kg body weight and per day, for 2 weeks.

### *Biological Samples*

(*a*) *Plasma samples*. Animals were bled from a caudal vessel using a heparinized syringe. Blood samples were taken just before the injection and 1, 3, 6, 9, 24, 48, 72, and 96 hr after the injection and then twice a week for 2 weeks. After centrifugation at 1500*g* for 20 min at 4°, the blood plasma were separated and frozen at −30° until assayed.

(*b*) *Perifusions*. Perifusions were run according to Bailhache *et al.* (1989). Briefly, the brain/pituitary was removed from the trout. A midsaggital section was made, passing through the third ventricle, leaving thus intact most hypothalamic structures. The half forebrain (including half a pituitary still connected) was then perifused (14°) with Hepes buffer (pH 7.4) in an appropriate chamber. After 3 hr of perifusion, sGnRH ($10^{-7}$ *M*) was added to the medium for 15 min. Fractions were collected every 2 min (flow rate: 1 ml per 5 min) and then frozen at −20° until assayed.

### *RIA Procedure*

Radioimmunological assays were run as previously described by Breton *et al.* (1978) using guinea pig IgG directed against the native sGtH II and rabbit IgG directed against guinea pig IgG. This double antibody RIA procedure has intraassay and interassay coefficients of variation (at 50% label binding) of 6.6 and 11%, respectively. The sensitivity of this RIA was 300 pg/ml sample.

### *ELISA Procedure*

An enzymoimmunometric assay was used (Ternynck and Avrameas, 1987). Briefly, hormone (reference sGtH II) coated onto the walls of microtiter plates competes for primary antibodies (anti-βsGtH II) with hormone which is free in solution. Appropriate steps reveal the immobilized sGtH II–antibody complexes. The "standard conditions" of our ELISA procedure, as used in this study, were:

*Step 1*. Two hundred microliters of reference sGtH II solution (2 ng/ml) in 0.05 *M* carbonate buffer (pH 9.6) was added to each well of a microtiter plate (NUNC IMMUNO II 96 F) and incubated for 2 hr at 37°.

*Step 2*. The low concentration of sGtH used in step 1 leaves many unbound sites which are saturated by unreactive proteins (nonimmunized sheep serum): 200 μl of PBS "S.T.G." (0.01 M phosphate buffer, pH 7.2, containing 0.15 *M* NaCl, 2% sheep serum, 0.05%, Tween 20, 25 mg/liter gentamincin sulfate) was added to each well. The plates were then incubated for 30 min at 37°.

*Step 3a*. Without preincubation: 20 to 100 μl of sample and 180 to 100 μl of primary antibody (final dilution: 1/175,000) were added to each well. The plates were then incubated for 4 hr at 37°.

*Step 3b*. With preincubation: 25 to 125 μl of sample and 225 to 125 μl of primary antibody (final dilution 1/175,000) were mixed in a tube and incubated at room temperature for 24 hr. Two hundred microliters of this solution was then added to the microplate wells and incubated for 2 hr at 37°.

*Step 4*. Two hundred microliters of goat anti-rabbit IgG from Jackson Immunoresearch (U.S.A.) (GAR 1/2000 in PBS S.T.G.) was added to each well. Plates were incubated at 37° for 30 min.

*Step 5.* Two hundred microliters of rabbit peroxidase–antiperoxidase from Nordic Immunology (The Netherlands) (rPAP 1/5000 in PBS S.T.G.) was added to each well. Plates were incubated at 37° for 30 min.

*Step 6.* Two hundred microliters of citrate phosphate buffer (0.15 *M*, pH 5.1) with orthophenylenediamine (OPD) (Sigma) (0.5 mg/ml) and 0.25 μl $H_2O_2$ (30%)/ml were added to each well and incubated at room temperature, in the dark for 1 hr.

*Step 7.* The enzymatic reaction was stopped by the addition of 50 μl of $H_2SO_4$ (2 *M*) to each well. After 20 min, the optical density was read with an automatic microplate reader MR 700 (Dynatech, Great Britain).

In each assay, the following was determined: Blanks = optical density (OD) of wells without coated GtH; $B_0$ = OD of wells without free GtH (maximum binding); $B$ = OD of wells with both coated and free GtH. All the wells received primary antibodies in equal quantity. The value of the blanks never exceeded 6% of the mean $B_0$ value. After steps 2, 3, 4, and 5, the wells were washed three times with 200 μl of phosphate buffer saline containing 0.05% of Tween 20.

A first protocol is described in the legend of Fig. 1. This protocol has been used to search for optimum conditions of the assay (experimental conditions of the seven steps have been studied one by one to elaborate the standard conditions).

### *Linear Transformation and Statistical Analysis*

After substraction of the mean blank value, $B/B_0$ values were submitted to the logit transformation: Logit $(B/B_0) = \log [B/B_0/(1 - B/B_0)]$. Linear regressions were then calculated by plotting the logit $(B/B_0)$ versus the ln(dose) of free hormone added to wells. Each biological sample was assayed in duplicate. Parallelism of each plasma dilution curve with the standard curve was tested by analysis of covariance. We also calculated the intraassay and interassay coefficients of variation of the ELISA procedure. Results of the *in vivo* experiments were statistically analyzed by Student's *t* test.

## RESULTS

### *Optimization of the ELISA Procedure*

In order to give a good precision to the assay, we searched for the optimum conditions of each step (i.e., optimum time and concentrations).

(*a*) *Kinetics* (*Fig. 1*). Time course studies were run for each step. sGtH binding to the wells was more rapid at 37° than at 4°. The amount of binding was also greater at 37° (Fig. 1a). Step 3 was relatively slow (Fig. 1b). In step 4, goat anti-rabbit (GAR) binding to the primary antibodies was extremely rapid: after 10 min, the reaction reached its maximum regardless of dilution rate (Fig. 1c). In step 7, we observed a great variability of optical densities between the $B_0$ wells when the plate was read immediately after $H_2SO_4$ addition (Fig. 1d). This variability decreased as optical densities reached their maximum value (Fig. 1d). To check if the acid concentration was suitable, we tried a 4 *M* $H_2SO_4$ solution which gave identical results.

For other time course steps, maximum binding levels of rPAP were observed after 20 min of incubation (step 5), optimal saturation after 30 min (step 2), and the highest level of substrate transformation by the peroxidase after 1 hr (data not shown).

(*b*) *Concentrations* (*Fig. 2*). Concentrations of coated GtH and primary antibody solution (steps 1 and 3) were investigated together. Primary antibody dilutions from 1/100,000 to 1/200,000 seemed to give good results (Fig. 2a). Therefore, when other parameters were optimized (see Materials and Methods: standard conditions), primary antibody dilutions were investigated in the range of 1/100,000 to 1/250,000. It appeared (Fig. 2b) that optical densities were not high enough with the 1 ng/ml coating solution, whereas 2 ng/ml gave high values (Fig. 2b).

Other antibodies were diluted to 1/2000 for GAR serum and to 1/5000 for rPAP complex. These concentrations gave good optical densities for $B_0$ and minimized nonspecific binding to the well. For saturation, the best results were obtained with 2% of sheep serum. In step 6, the highest observable value of $B_0$ was obtained by using 10 mg OPD and 5 μl $H_2O_2$ (30%) in 20 ml citrate phosphate buffer.

### *Standard Curves*

Standard curves were obtained by adding increasing doses of reference sGtH II in step 3.

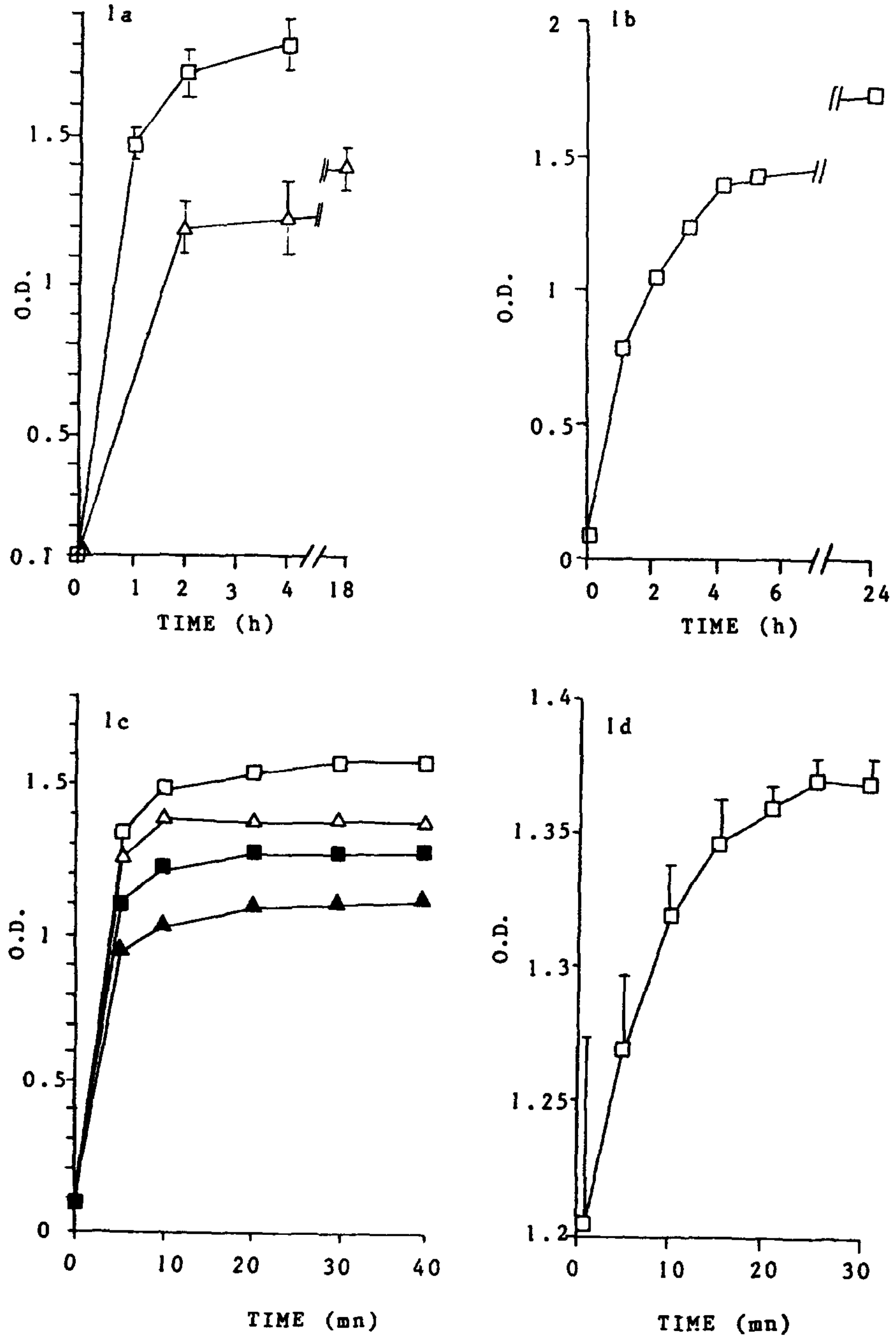


FIG. 1. Time course studies of different ELISA steps for optical density (OD) $B_0$ values. Experiments were done using a primary protocol which differed from the standard conditions: coating sGtH II 1.6 ng/ml vs 2 ng/ml; anti-βsGtH 1/80,000 vs 1/175,000; rPAP 1/2000 vs 1/5000; incubation time in step 6: 30 min vs 1 hr. (a) Step 1, at 37° (□) or at 4° (△), each point is the mean ± SD of six values. (b) Step 3. (c) Step 4, with different GAR dilutions: 1/1000 (□), 1/2000 (△), 1/5000 (■), and 1/10,000 (▲). (d) Step 7, each point is the mean ± SD of six values.

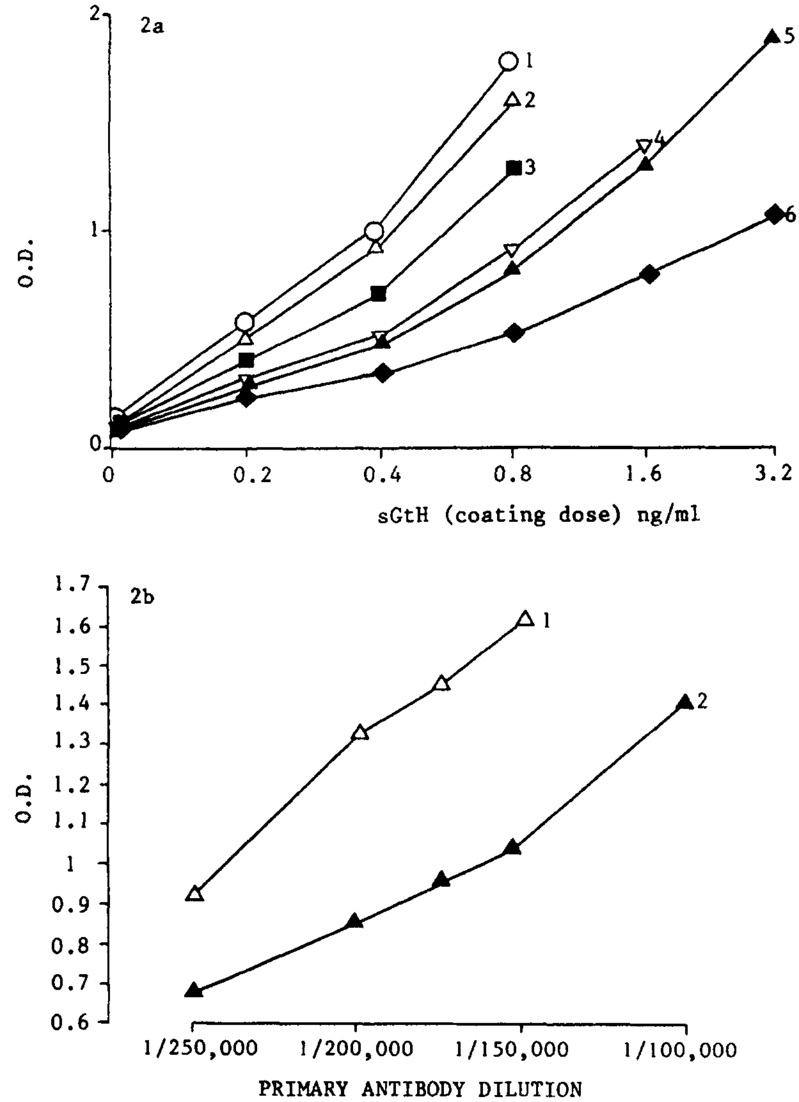


FIG. 2. Determination of the concentrations of coated sGtH II and primary antibodies. (a) $B_0$ evolution for increasing doses of coating with different anti-βsGtH serum dilutions: 1/10,000 (I), 1/20,000 (2), 1/40,000 (3), 1/80,000 (4), 1/100,000 (5), and 1/200,000 (6). For the other steps, the procedure was run as described in the legend of Fig. 1. (b) $B_0$ evolution for increasing anti-βsGtH serum concentrations with two different doses of coating: (1) 2 ng/ml and (2) 1 ng/ml. For other steps, the procedure was run as described under Materials and Methods.

The preincubation enhanced the sensitivity of the assay, as 90% bound corresponded to 7.28 pg/well of free hormone in the procedure involving a preincubation, whereas it was 24.3 pg/well without preincubation (Fig. 3) when incubation time in step 3 was the same for the two procedures.

Then, we studied the effect of different times of preincubation and incubation in step 3. Without preincubation, Fig. 4a shows that the equilibrium is reached after 4 hr of incubation. Thus, this procedure constitutes an equilibrium assay.

With a preincubation step, we can see that more or less than 2 hr of incubation induced a loss of sensitivity (Fig. 4b). It

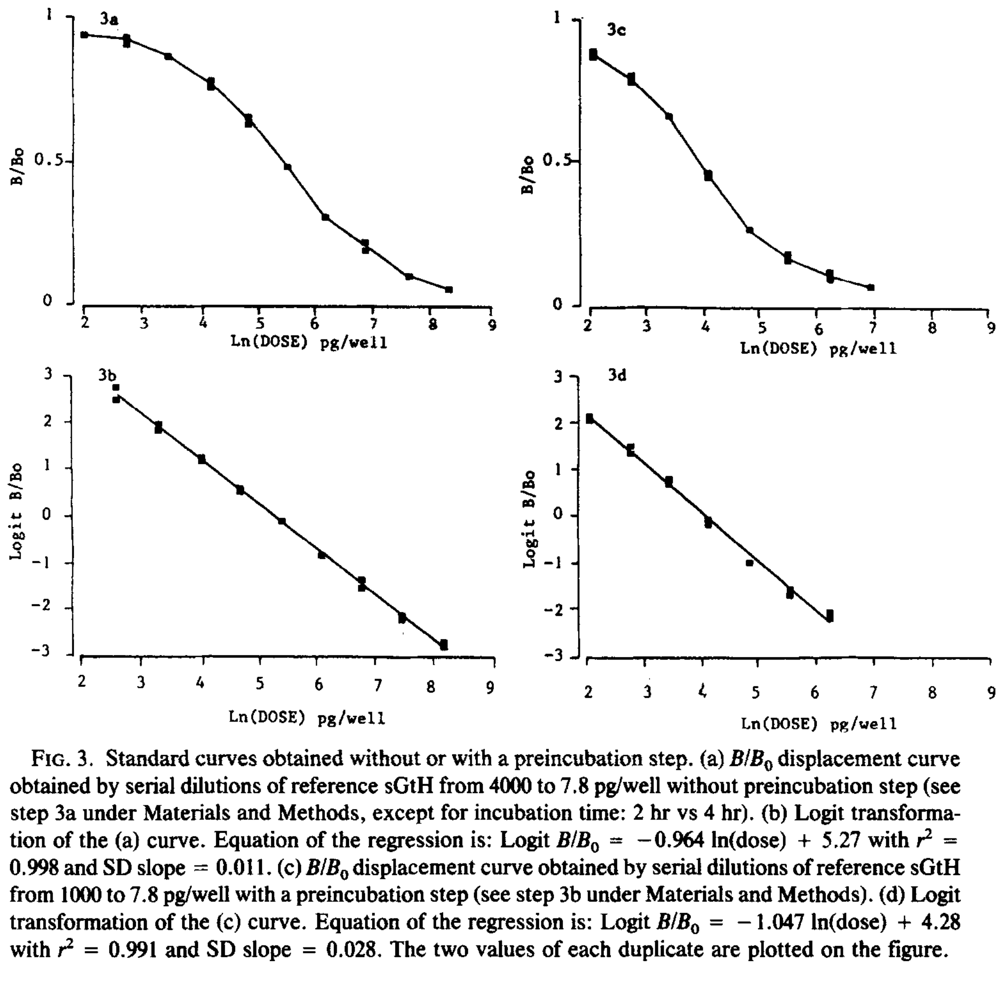


FIG. 3. Standard curves obtained without or with a preincubation step. (a) $B/B_0$ displacement curve obtained by serial dilutions of reference sGtH from 4000 to 7.8 pg/well without preincubation step (see step 3a under Materials and Methods, except for incubation time: 2 hr vs 4 hr). (b) Logit transformation of the (a) curve. Equation of the regression is: Logit $B/B_0 = -0.964 \ln(\text{dose}) + 5.27$ with $r^2 = 0.998$ and SD slope = 0.011. (c) $B/B_0$ displacement curve obtained by serial dilutions of reference sGtH from 1000 to 7.8 pg/well with a preincubation step (see step 3b under Materials and Methods). (d) Logit transformation of the (c) curve. Equation of the regression is: Logit $B/B_0 = -1.047 \ln(\text{dose}) + 4.28$ with $r^2 = 0.991$ and SD slope = 0.028. The two values of each duplicate are plotted on the figure.

appears in Fig. 4c that the equilibrium is reached after 24 hr of preincubation in the tube. This procedure, with only 2 hr of incubation in step 3, constitutes a nonequilibrium assay when compared to the equilibrium one described above.

### *GtH Determination in Biological Samples*

Serial dilutions of two plasma samples from mature female trout were assayed under both the equilibrium (Fig. 5a) and the nonequilibrium (Fig. 5b) procedures. In both cases, after logit transformation, the slopes of the regressions were not statistically different ($P > 0.25$); but GtH II contents of these plasmas were very different when assayed with the two procedures. This discrepancy also appeared when a constant amount of reference sGtH II was added in several dilutions of a plasma from an immature female trout. Measured doses were very close to the theoretical doses, only with a preincubation (Table 1). We did not notice any nonspecific binding in perifusion samples (results not shown).

### *Statistical Analysis*

Intraassay and interassay coefficients of variation have been calculated for biological samples ($n = 6$) around 50% bound, and they were 4.28 and 6.36%, respectively. We must also mention that the 36 outside wells were not found to be suitable for the assay when assayed samples gave about 90 or 10% bound values.

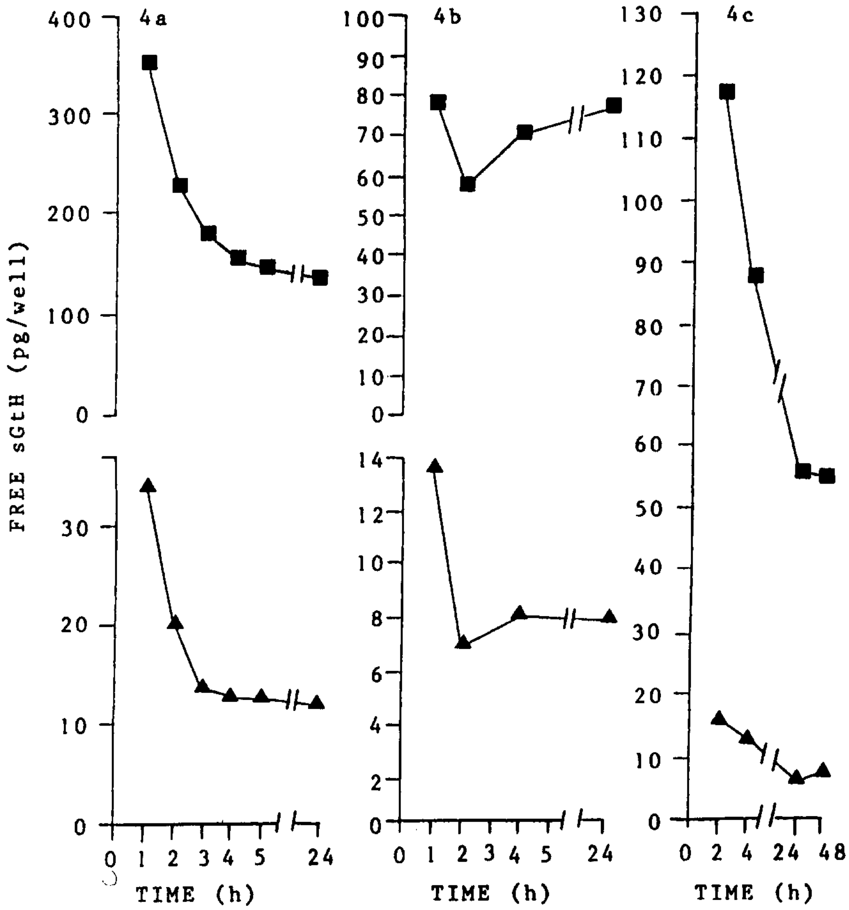


FIG. 4. Effect of incubation times in step 3 on the free sGtH doses necessary to obtain displacements of 50% (■) and 90% (▲). (a) Effect of incubation time in step 3a (Materials and Methods). (b) Effects of incubation time in step 3b, in a procedure with 24 hr of preincubation. (c) Effect of preincubation time in step 3b.

### *In Vivo Experiments*

No fish were found to ovulate between Day 0 and Day 4 after the beginning of the treatments. In the rainbow trout, it is known that plasma GtH levels increase after ovulation (Jalabert and Breton, 1980). After Day 4, in each group, there were both ovulated and unovulated females. For this reason, Fig. 6 only shows the evolutions of the plasma GtH levels during the time for which all the fish were at a similar physiological state.

In all groups, the GtH levels were similar at the beginning of the experiment and not statistically different (3.4 to 5.9 ng/ml). In the control group, they remained constant over the 4 days. But in all GnRHa-injected groups, the GtH levels increased significantly ($P < 0.005$), although the response varied according to the treatment.

In both native and slow-release forms, [D-Trp$^6$]LH-RH induced a similar stimulation of the GtH II secretion, peaking around 30 ng/ml as early as 1 hr after the injection. With both forms, the blood plasma GtH II levels similarly decreased 24 hr after treatment but they did not return to basal values in the group receiving the LH-RH slow-release form, in which they were maintained above 12 ng/ml. There was also the same phenomenon in animals treated with the microencapsulated [D-Arg[6],Pro[9] NHEt]sGnRH, although the initial stimulation was not as important.

In all treated groups, the mean blood plasma GtH levels increased again after Day 4, mainly correlated with the presence of ovulated females which ovulated for 80% of the population between Day 4 and Day 7; on the contrary, in the control group, the occurrence of ovulated females was spread over a longer period.

### *Perifused Half Brain/Pituitary Responsiveness to sGnRH*

Figure 7 represents the evolution of GtH levels (mean ± SEM, $n = 5$) in the perifusion medium of five experiments. As deter-

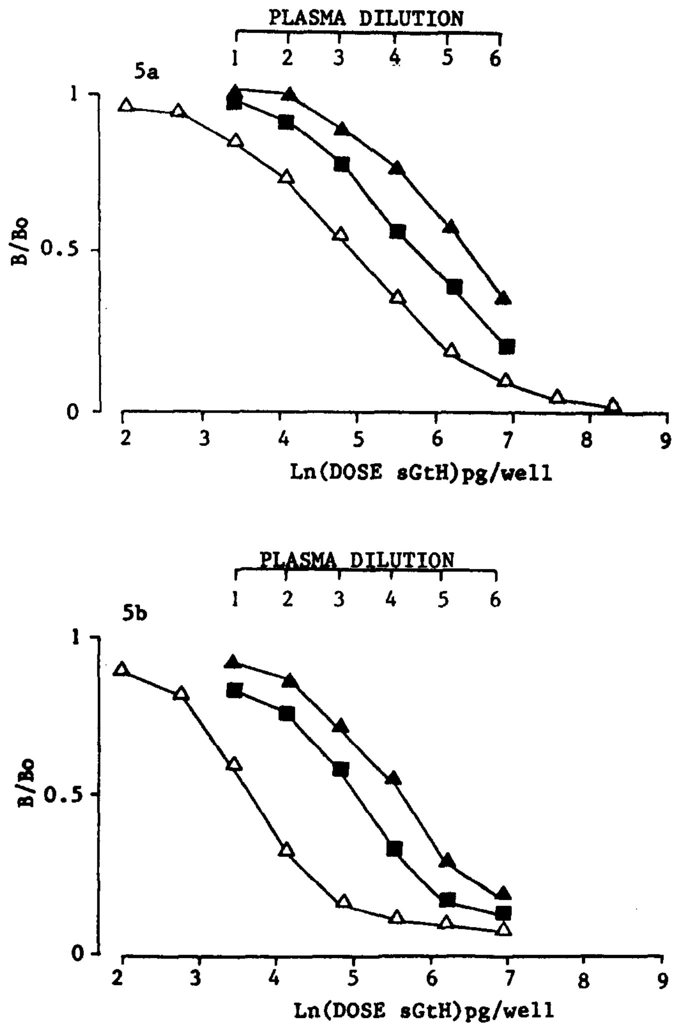


FIG. 5. Parallelism between standard curves and plasma sample dilution curves. (a) Displacement curves obtained with reference (△) sGtH II, (■) plasma from a rainbow trout at the oocyte maturation stage (mVG+), and (▲) plasma from a rainbow trout at the end of vitellogenesis (VG+), assayed under an equilibrium procedure. (b) Displacement curves obtained with reference (△) sGtH II and the same plasma samples as in (a), (■) plasma mVG+, and (▲) plasma VG+, assayed under a nonequilibrium procedure. Plasma dilutions were 1, 1/64; 2, 1/32; 3, 1/16; 4, 1/8; 5, 1/4, and 6, 1/2.

TABLE 1
COMPARISON BETWEEN THE ELISA GtH MEASUREMENTS AND THE THEORETICAL CONTENT OF DIFFERENT DILUTIONS OF A PLASMA SAMPLE FROM AN IMMATURE RAINBOW TROUT

| Dilution | Measured dose (pg/well) | Theoretical dose (pg/well) |
|---|---|---|
| Equilibrium procedure[a] | | |
| 1/85.3 | 326 | 334 |
| 1/42.6 | 383 | 351 |
| 1/21.3 | 467 | 385 |
| 1/10.6 | 595 | 453 |
| 1/05.3 | 842 | 590 |
| 1/02.6 | 1482 | 862 |
| Nonequilibrium procedure[b] | | |
| 1/85.3 | 241 | 252 |
| 1/42.6 | 243 | 254 |
| 1/21.3 | 231 | 258 |
| 1/10.6 | 282 | 267 |
| 1/05.3 | 273 | 284 |
| 1/02.6 | 298 | 317 |

*Note.* A first determination gave 1.45 ng/ml under the equilibrium procedure and 0.18 ng/ml under the nonequilibrium conditions.

[a] sGtH (317.5 pg/well) was added for each dilution of the plasma, when assayed under the nonequilibrium procedure. The theoretical dose (TD) is calculated as

$$\text{TD (pg/well)} = \frac{1.45 \cdot 10^3}{\text{dilution}} + 317.5$$

[b] sGtH (250 pg/well) was added for each dilution of the plasma, when assayed under the nonequilibrium procedure. TD is calculated as

$$\text{TD (pg/well)} = \frac{0.18 \cdot 10^3}{\text{dilution}} + 250.$$

mined by a two-way analysis of variance, GnRH application induced a significant transient increase of the GtH II release ($P < 0.05$). The nonequilibrium procedure allowed us to detect this rapid response to the sGnRH application, despite the low GtH II contents of these samples.

### *ELISA/RIA Correlation*

Plasma GtH concentrations were determined by both ELISA and RIA on 139 samples from animals treated with different analogs of the GnRH (Materials and Methods). A linear regression was calculated with the RIA dose vs the ELISA dose. We obtained the following equation:

$$\text{RIA dose} = 1.149 \text{ (ELISA dose)} - 0.09 \quad (r^2 = 0.942).$$

We made the same comparison with 27 samples from several perifusions, spreading over a wide range (from 0.5 to 100 ng/ml). Both RIA and ELISA (equilibrium procedure) were performed on those 27 samples. The linear regression, with RIA dose vs ELISA dose, gave the following equation:

$$\text{RIA dose} = 1.087 \text{ (ELISA dose)} + 0.52 \quad r^2 = 0.884).$$

## DISCUSSION

sGnRH and analogs of GnRH stimulate *in vivo* and *in vitro* GtH II secretion in salmonids. Microencapsulated or implanted analogs induce a more prolonged stimulation of the GtH II blood plasma levels. All of these observations are in agreement with those of Van der Kraak *et al.* (1983), Crim *et al.* (1983), Weil and Crim (1983), and Weil *et al.* (1986). The main purpose of this study, however, was to compare RIA and ELISA techniques for measuring GtH II levels in biological samples.

Some steps in our ELISA procedure (rPAP, primary antibody, OPD/$H_2O_2$) showed classical kinetics previously described by Signorella and Hymer (1984) and by Farrington and Hymer (1987), but other steps gave unusual results.

Our data show that it is necessary to wait for 20 min before reading the plate in step 7. As far as we are aware, there have been no papers recommending a delay between $H_2SO_4$ addition and the plate reading. Acid addition is usually admitted to stop reactions immediately. The modifications in the chromogen properties, appearing after acidification, are slower than normally thought.

The sensitivity and the range of our

ELISA procedure show that biological samples can be assayed under low dilution conditions (½). In this case (i.e., when using 100-μl samples), sensitivity (90% bound) is 70 pg/ml sample in the nonequilibrium procedure and 125 pg/ml sample in the equilibrium procedure. Even if the sensitivity of this equilibrium assay is not as high as the one of the nonequilibrium assay, it allows us to measure hormonal concentrations over a wider range and to carry out the entire procedure in 8 hr.

Our study demonstrates another important result: displacement curve parallelism is not an absolute criterion of validity for immunoassays. Indeed, analysis of covariance showed that the dilution curves paralleled the standard curves, whatever the procedure was (i.e., equilibrium and nonequilibrium procedures). The other experiment (addition of sGtH in plasma) revealed the equilibrium procedure to be unable to detect the right quantity of hormone. Only the nonequilibrium procedure gave good results. Why did the dilution curves parallel the standard curve in the equilibrium procedure whereas this procedure detected too high values of GtH II? One hypothesis could be that primary antibodies recognized another plasma molecule in the equilibrium procedure and that this molecule can displace antibody binding in a specific-like manner (i.e., with the same slope than for the standard curve). On the other hand,

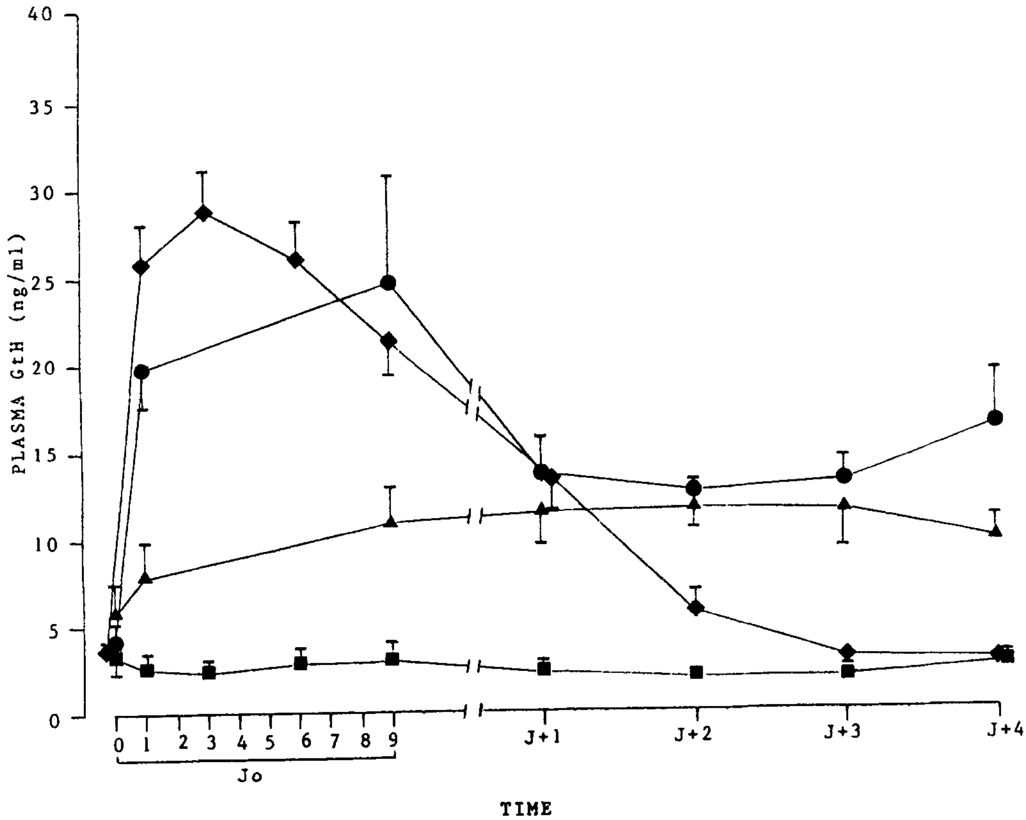


FIG. 6. Variations of the plasma tGtH II levels with different injections: (■) saline solution (control group); (♦) 20 μg/kg body weight of [D-Trp[6]]LH-RH; (●) 200 μg/kg body weight of slow-release form of [D-Trp[6]]LH-RH; and (▲) microencapsulated [D-Arg[6],Pro[9]NHEt]sGnRH. JO is the day of the treatment. Fish were sampled at 0, 1, 3, 6, and 9 hr at JO and 24 hr (J + 1), 48 hr (J + 2), 72 hr (J + 3), and 96 hr (J + 4) after. Each point is the mean ± SEM of six values.

in the nonequilibrium procedure, since anti-βGtH affinity may be greater for GtH II than for this other molecule, dissociation kinetics during incubation in step 3 may decrease cross-reactivity and increase specific detection of the gonadotropin.

*In vitro* experiments (with application of sGnRH to the preparation) as well as *in vivo* experiments (injections or pellet implantation of GnRH-a) allowed us to check the capacity of our procedure to detect a physiological response to a GnRH treatment. These results sustain the assumption that our assay really measures trout gonadotropin. [D-Trp$^6$]LH-RH (via native or slow-release form) evoked an immediate and more important release of GtH II than microencapsulated [D-Arg$^6$,Pro$^9$ NHEt] sGnRH, but these two analogs (except for native [D-Trp$^6$]LH-RH) induced similar GtH II levels in the days following the treatment. It is interesting that levels of GtH remained high in trout with the analog containing pellets. This could indicate that a constant high level of GnRH-a does not induce any "desensitization" at the pituitary level as reported by Zohar (1988) for another teleost, *Sparus aurata*. In the same way, continuous perifusion with [D-Ala$^6$,Pro$^9$ NHEt]LHRH induced high

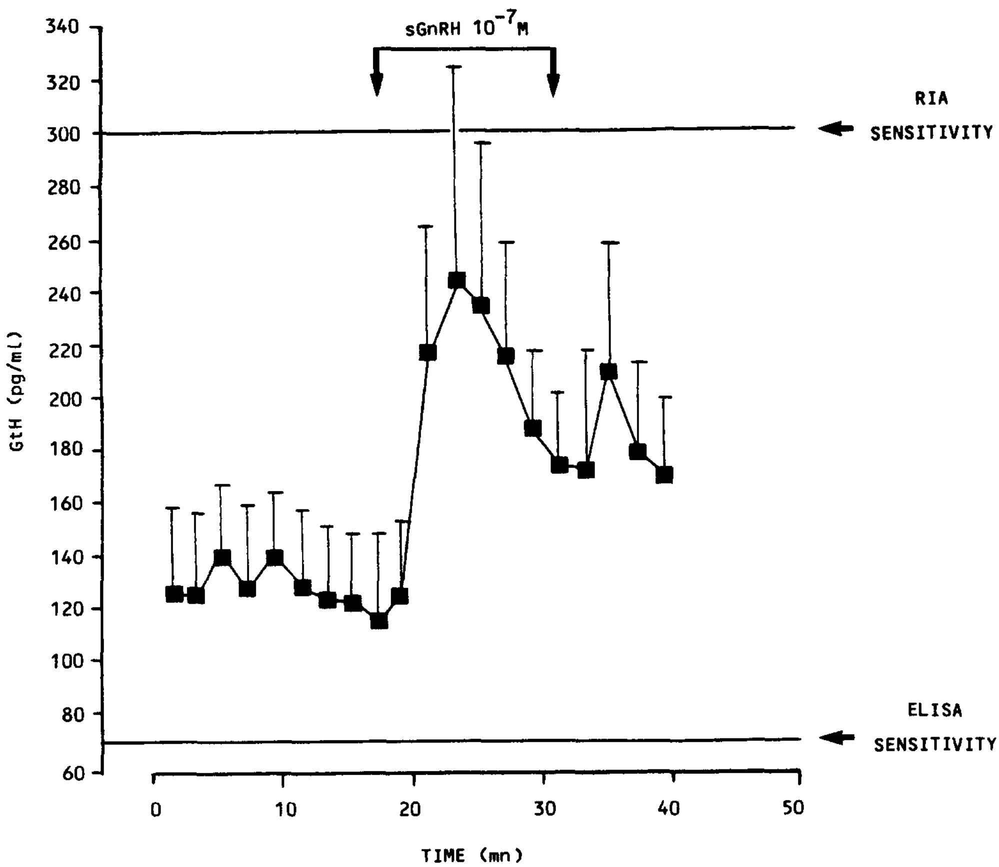


FIG. 7. tGtH II variations in the perifusion medium of a half brain/pituitary preparation. The preparation was stimulated by a 15-mn perifusion of sGnRH ($10^{-7}$ *M*). The half brain/pituitary was dissected from female rainbow trout at the early stage of vitellogenesis (oocyte diameter <0.5 mm). Minimum detectable doses of both RIA and ELISA are shown on the graph. Each point is the mean ± SEM of five values.

and constant levels of GtH release from Tilapia (Levavi-Zermonsky and Yaron, 1988) and from African catfish (De Leeuw *et al.*, 1986) pituitary fragments.

Our perifusion chamber is specially adapted to the brain/pituitary preparation and allows both local administrations of bioactive molecules in specific hypothalamic nuclei and electrophysiological recordings (Bailhache *et al.*, 1989). The GtH levels reported here are lower than can be seen in literature and must be due to the integrity of the half pituitaries used in these studies. However, the results obtained with our perifusion technique showed that sGnRH is able to induce a twofold transient increase of GtH II release in early vitellogenic trout. This weak pituitary responsiveness is in agreement with previous studies of Weil *et al.* (1978). The minimum detectable dose of each method (RIA and ELISA) is shown in Fig. 7 and clearly shows the value of our method. The optimal RIA sensitivity is 300 pg/ml but, in a majority of cases, this assay allows only about 1 ng/ml of sensitivity. Thus, when perifusion experiments are performed with immature trout pituitaries, an unobserved response to a GnRH treatment may be misunderstood by using the RIA technique. On the other hand, our ELISA procedure is of great interest in view of pulsatility detection and experiments on juvenile fish.

In conclusion, we have developed an ELISA procedure that allows a specific heterologous assay of *S. gairdneri* GtH II from purified salmon gonadotropin. This new reliable assay meets the general quality criteria required for an immunological assay. The good agreement between the GtH II concentrations determined by ELISA and those determined by RIA indicates that this new assay can replace RIA in other ongoing studies since sensitivity is improved (70 pg/ml against 300 pg/ml in RIA), assay characteristics are more repeatable (e.g., no loss of specific activity as in RIA), and it is performed quicker than RIA (8 to 26 hr against 3 to 5 days).

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