Fish Physiology and Biochemistry vol. 14 no. 6 pp 489-500 (1995)
Kugler Publications, Amsterdam/New York

Involvement of gonadal steroids in final oocyte maturation of white perch (*Morone americana*) and white bass (*M. chrysops*): *in vivo* and *in vitro* studies

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Accepted: June 4, 1995

Keywords: gonad, maturation, *Morone*, oocyte, ovary, perciform, steroidogenesis, steroids, teleost

Abstract

Plasma estradiol-17β (E_2), testosterone (T), 17α,20β-dihydroxy-4-pregnen-3-one (DHP) and 17α,20β,21-trihydroxy-4-pregnen-3-one (20β-S) levels were measured by radioimmunoassay (RIA) in white perch (*Morone americana*) and white bass (*M. chrysops*) that were induced to undergo final oocyte maturation (FOM) with human chorionic gonadotropin (hCG). Plasma DHP levels increased in females of both species in association with oocyte germinal vesicle migration (GVM) and germinal vesicle breakdown (GVBD) and decreased thereafter. Plasma 20β-S levels also increased with oocyte GVM in white bass, but were several-fold lower than DHP levels. Circulating E_2 and T levels were greatest during GVM and GVBD in both species and decreased to low levels during oocyte hydration and ovulation. Follicles from white perch and white bass which received a priming injection of hCG *in vivo*, produced both DHP and 20β-S *in vitro* after exposure to hCG and their oocytes underwent GVBD. Ovarian incubates from unprimed fish of either species produced only E_2 and T and their oocytes did not complete GVBD. Oocytes from unprimed bass, but not perch, matured when follicles were exposed to hCG *in vitro*. Both trilostane and cycloheximide blocked *in vitro* production of DHP and 20β-S and oocyte GVBD by white perch follices. DHP and 20β-S were equipotent inducers of FOM in the GVBD bioassay. None of several other structurally-related steroids tested were effective within a physiological range of concentrations. These results indicate a role for DHP and 20β-S in the control of FOM in white perch and white bass.

Introduction

During ovarian growth and development in teleost fish, gonadotropin (GTH) stimulates follicular production of estradiol-17β (E_2) and its precursor, testosterone (T) (Fostier *et al.* 1983). In post-vitellogenic follicles, GTH stimulates a change in steroidogenesis from primarily E_2 and T production toward synthesis of a C_{21} maturation-inducing steroid hormone (MIH) which mediates final oocyte maturation (FOM). In salmonids, the MIH is 17α,20β-dihydroxy-4-pregnen-3-one (DHP) (Nagahama and Adachi 1985; Nagahama 1987a), a steroid implicated as the MIH in several other teleosts as well (Scott and Canario 1987). In sciaenids, 17α,20β,21-trihydroxy-4-pregnen-3-one (20β-S) has been identified as the MIH (Trant *et al.* 1986; Thomas and Trant 1989; Trant and Thomas 1989; Patino and Thomas 1990a). It has also been associated with the control of FOM in some other

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perciforms (Thomas 1988; Asahina *et al.* 1991; Modesto and Canario 1993; King *et al.* 1994a,b). DHP and 20β-S are the most potent inducers of oocyte germinal vesicle breakdown (GVBD) in bioassays using follicles from a variety of teleosts (Goetz 1983; Scott and Canario 1987).

Fully grown oocytes of some teleosts are not competent to respond to MIH unless they have been previously exposed to or 'primed' by GTH (Kobayashi *et al.* 1988; Zhu *et al.* 1989; Patino and Thomas 1990a,b; Kagawa *et al.* 1994). This priming effect can be induced by exposure to human chorionic gonadotropin (hCG) *in vivo* or *in vitro* (Patino and Thomas 1990b). In Atlantic croaker (*Micropogonias undulatus*) and red seabream (*Pagrus major*), priming appears to be dependent on protein synthesis (Patino and Thomas 1990b; Kagawa *et al.* 1994). In spotted seatrout (*Cynoscion undulatus*), it is associated with increases in 20β-S receptors on ovarian membranes (Patino and Thomas 1990c; Thomas and Patino 1991).

Previous studies of striped bass (*Morone saxatilis*), a species that recruits and spawns a single batch of eggs annually, showed that plasma DHP and 20β-S levels increased as levels of E_2 and T decreased in fish undergoing FOM (Berlinsky and Specker 1991; King *et al.* 1994a). Similarly, both DHP and 20β-S were produced by striped bass ovarian fragments undergoing hCG-induced GVBD *in vitro*, while production of E_2 and T declined (King *et al.* 1994b). To further understand the endocrine control of FOM in temperate basses (genus *Morone*), we investigated changes in gonadal steroid hormone levels accompanying FOM *in vivo* and *in vitro* in white perch (*M. americana*) and white bass (*M. chrysops*). Unlike striped bass, mature females of these species are multiple-clutch batch spawners with ovaries containing follicles at all stages of growth and maturation.

The objectives of the present study were to: 1) relate changes in plasma levels of DHP, 20β-S, E_2 and T to specific stages of oocyte maturation in females undergoing hCG-induced FOM, 2) evaluate the effects of a priming injection of hCG on *in vitro* ovarian steroidogenesis and FOM, 3) provide preliminary data on the bioactivity of DHP and 20β-S for inducing GVBD, and 4) test the effects of inhibitors of steroidogenesis and protein synthesis on the ability of hCG to induce DHP and 20β-S production and FOM in isolated ovarian fragments.

Materials and methods

Chemicals

Authentic DHP and 20β-S were purchased from Steraloids, Inc. (Wilton, NH). The hCG was purchased from Steris Laboratories (Phoenix, AZ). The 3β-hydroxy-Δ^5-steroid dehydrogenase (3β-HSD) inhibitor, trilostane, was a gift from Sterling Drug Inc. (Rensselaer, NY). All other steroids were purchased from Sigma Chemical Co. (St. Louis, MO). Culture media reagents and solvents were purchased from Fisher Scientific (Pittsburgh, PA). Cycloheximide was dissolved directly into incubation media. Trilostane was dissolved in dimethyl sulfoxide (DMSO) and steroids were prepared in methanol. Carrier solvents never exceeded 0.1% (v/v) in the culture media.

Animals

Adult white perch (range 130–265 g body weight) were obtained from broodstocks maintained at the Pamlico Aquaculture Field Laboratory of North Carolina State University (Jackson and Sullivan 1995). They were held in the laboratory in 1600 liter circular fiberglass tanks which were part of a recirculating water system (total volume 11,000 l) containing dechlorinated municipal water and fitted with 2 external biological filters. Hardness and alkalinity of the system water were maintained (range = 150–200 mg l^{-1}) by adding synthetic sea salts (Instant Ocean; Aquarium Systems, Mentor, OH) and sodium bicarbonate.

Entrainment of the white perch reproductive cycle was accomplished by timer-controlled fluorescent lighting of either 9L:15D (short) or 15L:9D (long) days. Thirty minutes of simulated dawn or dusk was provided with an incandescent 60W bulb coupled to a separate timer. Water temperature was maintained at 10–14°C during the short-day re-

gime and 15–25°C during the long-day regime. A 3 hp water chiller (Aquanetics; San Diego, CA) and a 3000 watt in-line water heater (Vulcan Electric; Kezar Falls, ME) were utilized to adjust water temperature as necessary. Following the spawning season, fish were maintained in reproductively-regressed condition for approximately 1 month (long day and 25°C). To initiate ovarian growth, water temperature was decreased to 13 ± 1°C over a 2–3 week period and photoperiod was shifted from long to short days. After 6–8 weeks, and at monthly intervals thereafter, ovarian biopsy samples were obtained with fire-polished microhematocrit tubes to monitor ovarian development. Follicle diameters were measured with a dissecting microscope and ocular micrometer to determine the status of ovarian maturation. Once vitellogenesis had been initiated (maximum follicle diameter >250 μm; Jackson and Sullivan 1995), the photoperiod was shifted from short to long days to provide appropriate conditions for rapid ovarian growth. When the largest follicles approached the size when they become competent to complete hCG-induced FOM *in vivo* (maximum follicle diameter ≥560 μm; W. King V, *unpublished*), water temperature was increased to 19 ± 1°C for at least 5 days prior to the initiation of experiments.

Wild white bass (range 340–440 g body weight) with fully-grown ovaries (maximum follicle diameter = 600 μm) were obtained from a commercial hybrid striped bass farm (Carolina Fisheries; Aurora, NC) where they had been maintained for several weeks after capture in 1600 liter circular tanks under long days (>16 h light) at 12°C. They were introduced into the recirculating water system described above and water temperature was increased from 12–19°C during the next 5 days prior to the initiation of experiments.

Induction of final oocyte maturation in vivo

White perch eligible for induction of FOM with hCG, as determined by the diameter of their most advanced follicles, were anesthetized in quinaldine sulfate (15 mg l^{-1}; Argent Chemical Co., Redmond, WA), weighed, and an ovarian biopsy sample was taken as described above. Biopsy samples were chemically-cleared in fixative (ethanol:formalin: acetic acid; 6:3:1, v/v) to determine the position of the oocyte germinal vesicle (GV) at the beginning of the experiment (King *et al.* 1994a). Animals were identified with colored streamer tags (Floy Manufacturing; Seattle, WA) and injected with hCG (330 IU kg^{-1} body weight i.m.). At 24, 30, 38, and 44 h after hormone injection, fish were anesthetized and an ovarian biopsy sample was obtained to determine the stage of oocyte maturation. Since the progression of FOM was not synchronous among individuals, fish were sampled for blood based on the position of the GV in their most mature oocytes as FOM progressed. Blood samples were collected by caudal puncture with heparinized syringes. Plasma was isolated by centrifugation and stored at −80°C until analysis of steroid hormone concentration by radioimmunoassay (RIA). White bass with fully-grown ovaries (maximum follicle diameter = 600 μm) were induced to undergo FOM with hCG as described above for perch, but blood samples were obtained from them at 0, 21, 29, 35, and 41 h after hCG injection.

Bioassay of final oocyte maturation in vitro

Several females of each species with fully-grown ovaries were injected with hCG (100 IU kg^{-1} body weight; primed) or vehicle alone (0.9% NaCl; unprimed). After 18 h, and at approximately 8 h intervals thereafter, the fish were anesthetized and an ovarian biopsy sample was taken and chemically cleared to determine the location of the oocyte GV in the largest follicles. When the largest oocytes in primed females showed coalescence of lipid in the ooplasm surrounding a centrally-located GV (CGV), the fish were killed in concentrated anesthetic (MS–222). Ovaries were excised from primed and unprimed fish, split lengthwise, and placed into cold (4°C) Cortland's balanced physiological saline during transport to the laboratory. Ovaries were then placed into Petri dishes (15 × 150 mm) containing fresh media (4°C) and fragmented with scissors and fine forceps (King *et al.* 1994b). Quantities of ovarian fragments appropriate for a particular ex-

periment (50–250 mg wet wt) were added to 24-well culture plates containing media with or without various concentrations of hCG, steroid hormones or inhibitors. All ovarian fragments were preincubated for 1 h in fresh media prior to the initiation of experiments.

White perch ovarian fragments cultured with inhibitors were preincubated an additional 1 h with the inhibitor prior to the addition of hCG (5 IU ml^{-1}). Most cultures were performed in 1 ml of medium in triplicate. In experiments examining the time course of ovarian steroidogenesis and FOM, incubations contained 6 replicates of stimulated (+hCG) and 2 replicates of control (no hCG) ovarian fragments each in 2 ml of medium. Incubates were placed into a Dubnoff shaking incubator at 22°C under air for 21–66 h. Upon termination of the experiment, medium was aspirated, centrifuged, and stored at −80°C for later measurement of steroid hormone concentration by RIA. Ovarian fragments from all experiments were chemically cleared, examined using a stereomicroscope, and then scored for the percentage of oocytes that had completed GVBD. In addition to undergoing GV migration and GVBD, maturing oocytes of white perch and white bass increase in diameter as the ooplasm clears while prematurational follicles remain opaque. Opaque CGV-stage follicles were considered to be vitellogenic and incapable of FOM. Only oocytes that were maturing or had completed GVBD at the termination of the culture were included in the scoring.

Radioimmunoassay

Steroids were double extracted or triple extracted (T only) from aliquots of plasma or culture medium (100 µl for DHP and 20β-S; 50 or 100 µl for E_2 and 15 µl for T) with ethyl ether and dried under a stream of nitrogen gas at 37°C. Residues were resuspended in 200 µl of RIA buffer and measured by RIA as previously described for E_2 and T (Woods and Sullivan 1993) or for DHP and 20β-S (King *et al.* 1994a). The steroid RIA's were validated for use with both white bass and white perch plasma and culture media (King *et al.* 1994b). Extracts of plasma or media pools diluted parallel with authentic standards over a range from 15–100 µl. Recoveries of authentic E_2, T, DHP or 20β-S from media and plasma extracts were >90% for all assays for both species.

Data analysis

Prior to statistical analysis, data for plasma steroid levels in white perch were separated into 3 groups based on the location of the GV in the most mature oocytes: 1) germinal vesicle migration (GVM), 2) GVBD, and 3) hydration with or without ovulation (HYDR). Comparable data for white bass were similarly separated with the addition of a fourth group of samples obtained just prior to hCG injection (prematuration, PM). For *in vitro* studies, cultures were performed using ovaries from at least 2 fish, but the data presented in each figure were collected using an ovary from one animal. Significant differences between mean steroid hormone levels in culture media or blood plasma or between mean percentages of oocytes undergoing GVBD *in vitro* were routinely identified by one-way analysis of variance (ANOVA) followed by Duncan's New Multiple Range Test (Zar 1974) using statistical software for the microcomputer (SUPERANOVA, Abacus Concepts, Berkeley, CA). The *a priori* level of statistical significance for all tests was $p \leq 0.05$. A two-way ANOVA was used to evaluate the relationship between steroid structure and GVBD-inducing potency shown in Figure 7.

Results

Plasma levels of gonadal steroids during FOM

Plasma DHP levels were significantly greater in white perch females sampled with oocytes that had completed GVBD as compared to levels seen in animals whose most mature oocytes were undergoing GVM (Fig. 1). Plasma DHP levels then decreased significantly with oocyte hydration and ovulation to low levels similar to those associated with GVM. Plasma 20β-S levels were detectable in

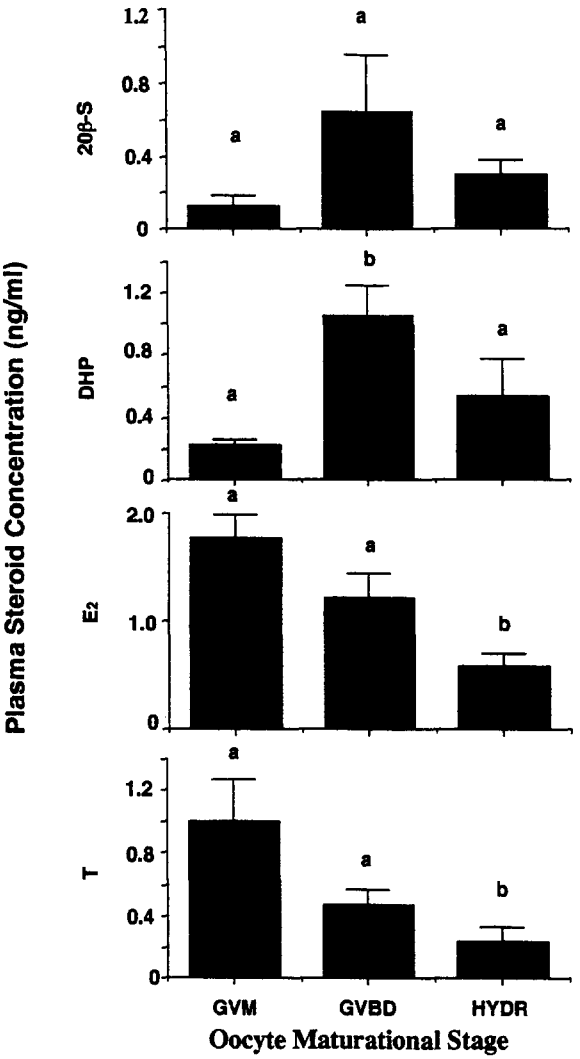

Fig. 1. Plasma levels of 17α,20β,21-trihydroxy-4-pregnen-3-one (20β-S), 17α,20β-dihydroxy-4-pregnen-3-one (DHP), estradiol-17β (E_2), and testosterone (T) in white perch injected with hCG (330 IU kg^{-1} body weight) and sampled when their largest oocytes were in various stages of final oocyte maturation. GVM (n = 15), germinal vesicle migration; GVBD (n = 7), germinal vesicle breakdown; HYDR (n = 9), hydration stages following GVBD including ovulation. Bars with different letter superscripts indicate mean values that are significantly different ($p < 0.05$). Vertical brackets represent the SEM.

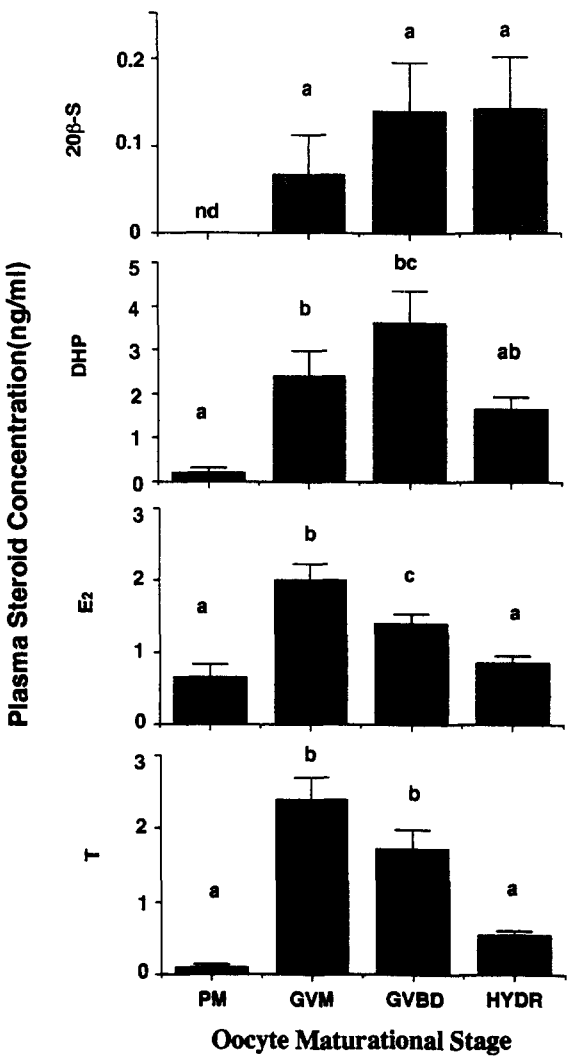

Fig. 2. Plasma levels of 17α,20β,21-trihydroxy-4-pregnen-3-one (20β-S), 17α,20β-dihydroxy-4-pregnen-3-one (DHP), estradiol-17β (E_2), and testosterone (T) in white bass injected with hCG (330 IU kg^{-1} body weight) and sampled when their largest oocytes were in various stages of final oocyte maturation. PM (n = 4), pre-maturation (at injection); GVM (n = 8), germinal vesicle migration; GVBD (n = 9), germinal vesicle breakdown; HYDR (n = 9), hydration stages following GVBD including ovulation. Bars with different letter superscripts indicate mean values that are significantly different ($p < 0.05$). Vertical brackets represent the SEM.

white perch sampled with oocytes undergoing GVM and tended to rise in association with oocyte GVBD, but the increase was not statistically significant. Plasma E_2 and T levels were highest in females whose most mature oocytes were undergoing GVM; they then decreased significantly to reach their lowest levels coinciding with oocyte hydration and ovulation.

Similar patterns in the circulating levels of gonadal steroid hormones were observed during hCG-induced FOM of white bass (Fig. 2). Plasma DHP,

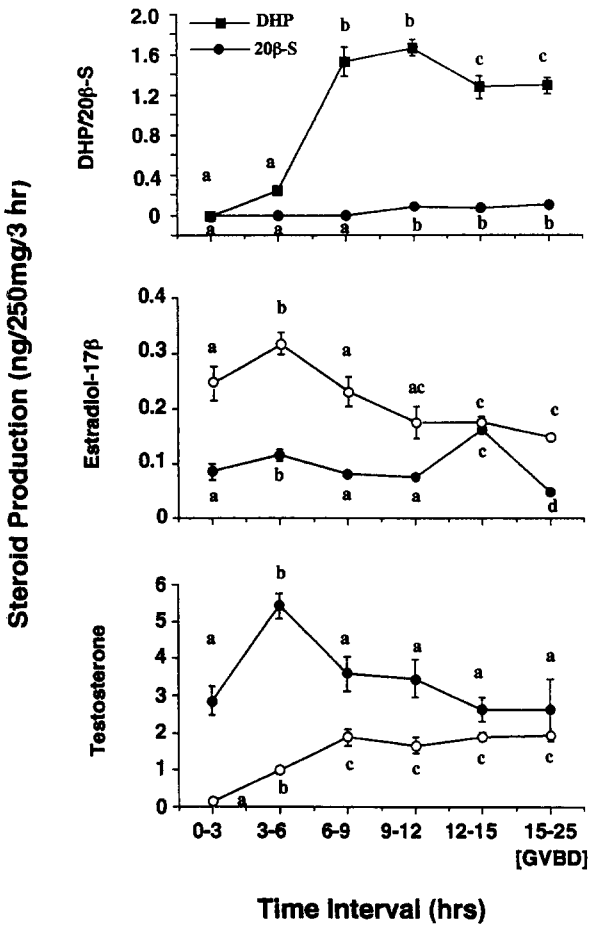

Fig. 3. In vitro time course of 17α,20β-dihydroxy-4-pregnen-3-one (DHP), 17α,20β,21-trihydroxy-4-pregnen-3-one (20β-S), estradiol-17β, and testosterone production by ovarian fragments (250 mg) from unprimed or primed white perch. Priming was accomplished by injecting the fish with hCG (100 IU kg^{-1} body weight, im) approximately 24 h prior to collecting ovarian tissue for the incubations. Unprimed fish were injected with vehicle alone (0.9% NaCl). Culture media (2 ml) containing 25 IU hCG ml^{-1} was removed and replaced every 3 h. Filled (primed) and unfilled circles or squares (unprimed) represent the mean steroid concentration for n = 6 incubations with hCG. Vertical brackets represent the SEM. Media from incubates containing unprimed fragments had non-detectable levels of DHP and 20β-S when incubated with hCG and did not undergo germinal vesicle breakdown (GVBD) (data not shown). Symbols with different letter superscripts or subscripts indicate mean values that are significantly different ($p < 0.05$).

20β-S, E_2 and T were at their lowest levels in the four fish sampled just prior to hCG injection (PM). Maximum plasma DHP levels were associated with oocyte GVM and GVBD. DHP levels then decreased with oocyte hydration and ovulation. Although plasma 20β-S levels appeared to increase initially in white bass undergoing oocyte GVM, the change was not statistically significant. Plasma E_2 levels increased with oocyte GVM and then decreased significantly with oocyte GVBD and hydration. Plasma T levels also increased significantly with oocyte GVM and then decreased significantly during oocyte hydration to the same low levels observed at the time of hCG injection (PM).

Time course of steroidogenesis during FOM in vitro

In cultures containing primed white perch ovarian fragments, DHP production was low during the first two 3 h incubation intervals, reached a peak during the interval from 6–9 h of incubation, and was sustained at high levels thereafter (Fig. 3). 20β-S production rose slightly but significantly after the 9–12 h incubation interval in cultures containing primed ovarian fragments. Approximately half of the oocytes in primed incubates underwent GVBD during the 12–15 h interval and by hour 25, most (75%) had completed GVBD. Levels of DHP and 20β-S were non-detectable in pooled samples of media (with or without hCG) from all time intervals that contained unprimed white perch ovarian fragments. The oocytes in these cultures did not undergo GVBD (data not shown).

Production of E_2 by ovarian fragments from primed white perch increased slightly during the 3–6 h incubation period and then returned to initial levels during hours 6–9 and 9–12 of incubation (Fig. 3). E_2 production was maximal during the interval from 12–15 h of incubation and then decreased thereafter to its lowest level. Production of E_2 by ovarian fragments from unprimed white perch was 2–2.5 × greater than that by primed fragments during the first 12 h of incubation. Similar to the primed incubates, unprimed incubates secreted significantly more E_2 during hours 3–6 as compared to the initial interval. However, E_2 production by unprimed incubates then decreased steadily for the remainder of the experiment and no peak during hours 12–15 was seen. A pooled sample of control media (no hCG) from all time

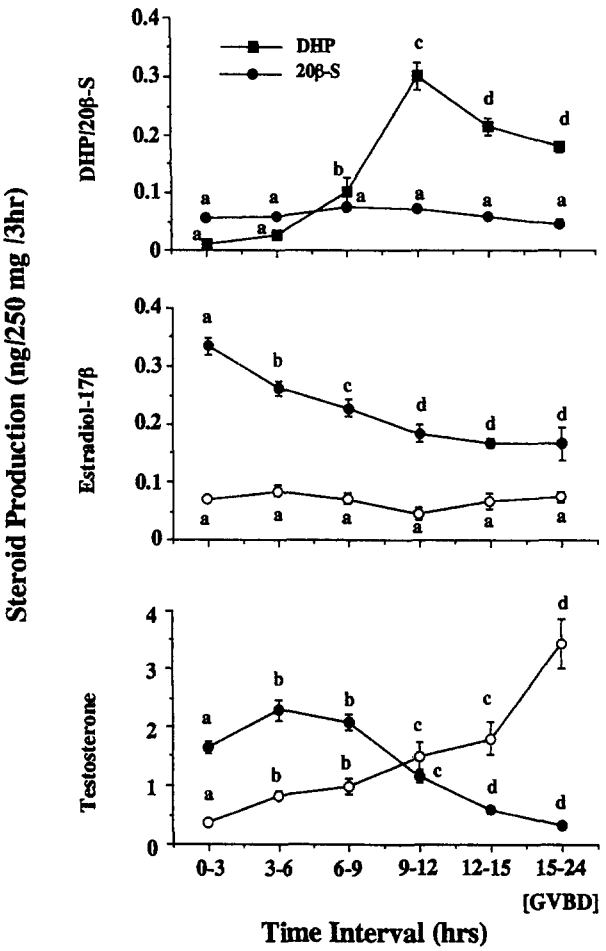

Fig. 4. In vitro time course of 17α,20β-dihydroxy-4-pregnen-3-one (DHP), 17α,20β,21-trihydroxy-4-pregnen-3-one (20β-S), estradiol-17β, and testosterone production by ovarian fragments (250 mg) from unprimed or primed white bass. Priming was accomplished by injecting the fish with hCG (100 IU kg^{-1} body weight; im) approximately 24 h prior to collecting ovarian tissue for the incubations. Unprimed fish were injected with vehicle alone (0.9% NaCl). Culture media (2 ml) containing 25 IU hCG ml^{-1} was removed and replaced every 3 h. Filled (primed) and open symbols (unprimed) represent the mean steroid concentration for n = 6 incubations with hCG. Media from incubates containing unprimed fragments had non-detectable levels of DHP and 20β-S when incubated with hCG and did not undergo germinal vesicle breakdown (GVBD) although their oocytes matured to an advanced stage (germinal vesicle migration, GVM) of maturation (data not shown). Symbols with different letter superscripts or subscripts indicate mean values that are significantly different ($p < 0.05$).

intervals which contained primed perch incubates produced little E_2 (0.08 ± 0.01 ng^{-1} mg^{-1}). Similar cultures containing unprimed incubates had non-detectable levels of E_2 in the medium (data not shown).

Production of T by primed white perch ovarian fragments increased substantially during hours 3–6 of incubation as compared to the initial interval 0–3 h) (Fig. 3). T production then declined to initial levels where it remained for the duration of the experiment. T production by unprimed ovarian fragments of white perch was at its lowest during 0–3 h of incubation and increased steadily and significantly later in the experiment to reach its greatest level at 6–9 h and remained elevated for the duration of the experiment. A pooled sample of control media (no hCG) from all time intervals which contained primed perch incubates produced substantial levels of T (1.6 ng mg^{-1} h^{-1}) while unprimed control incubates produced low levels of T (0.11 ± 0.01 ng mg^{-1} h^{-1}) (data not shown).

The *in vitro* time course of steroidogenesis by ovarian fragments from primed and unprimed white bass is shown in Figure 4. DHP production was nearly non-detectable in cultures of primed incubates through hour 6 of incubation, and then increased significantly during 6–9 h reaching a peak during hours 9–12, decreasing only slightly thereafter. 20β-S production by primed ovarian incubates was low but detectable during the entire experiment. Oocytes initiated GVBD during hours 12–15 and most (95%) had undergone GVBD after 24 h of incubation. Although oocytes from unprimed females did not undergo GVBD *in vitro*, most progressed to an advanced stage of GVM in the presence of hCG. Levels of DHP and 20β-S were non-detectable in pooled samples of media (with or without hCG) from all time intervals that contained unprimed white bass ovarian fragments (data not shown).

Production of E_2 by ovarian fragments from primed white bass was greatest during 0–3 h of incubation and then decreased continuously until the end of the 9–12 h incubation interval, remaining constant thereafter (Fig. 4). In contrast, E_2 production by unprimed incubates was sustained at low but detectable levels throughout the experiment. A pooled sample of control media (no hCG) from all time intervals that contained either primed or unprimed bass incubates produced low levels of

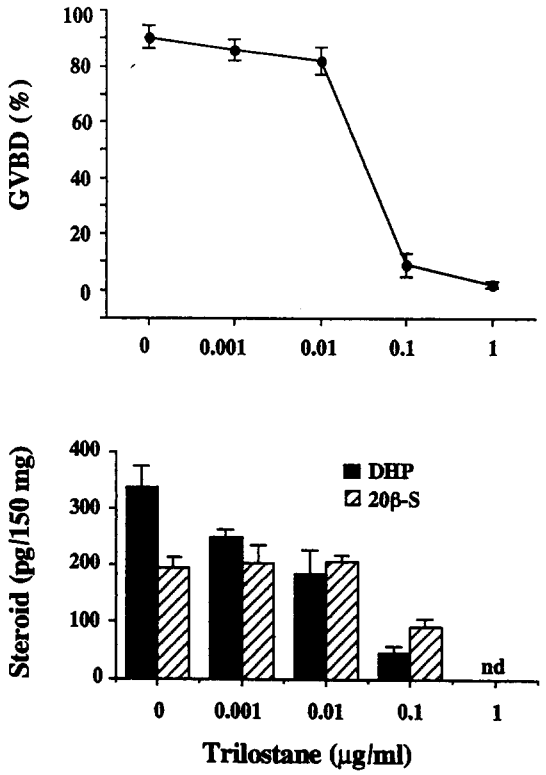

Fig. 5. The percentage of oocytes completing germinal vesicle breakdown (GVBD) and steroid hormone (17α,20β-dihydroxy-4-pregnen-3-one, DHP; 17α,20β,21-trihydroxy-4-pregnen-3-one,20β-S) production by ovarian fragments (150 mg ml^{-1}) of white perch incubated with 5 IU hCG ml^{-1} and various concentrations of the 3β-hydroxy-Δ^5-steroid dehydrogenase inhibitor trilostane. Cultures were preincubated with trilostane for 1 h prior to the addition of hCG. Bars indicate the mean value for n = 3 incubations. Vertical brackets indicate SEM. nd = non-detectable.

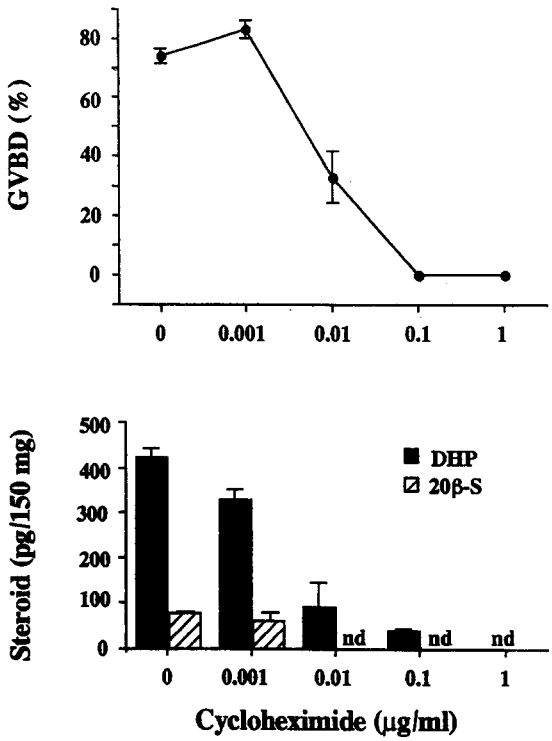

Fig. 6. The percentage of oocytes completing germinal vesicle breakdown (GVBD) and steroid hormone (17α,20β-dihydroxy-4-pregnen-3-one, DHP; 17α,20β,21-trihydroxy-4-pregnen-3-one, 20β-S) production by ovarian fragments (150 mg ml^{-1}) of white perch incubated with 5 IU hCG ml^{-1} and various concentrations of the translation inhibitor cycloheximide. Cultures were preincubated with cycloheximide for 1 h prior to the addition of hCG. Bars indicate the mean value for n = 3 incubations. Vertical brackets indicate SEM. nd = non-detectable.

E_2 (0.31 ± 0.07 ng mg^{-1} h^{-1}, primed; 0.12 ± 0.03 ng mg^{-1} h^{-1}, unprimed) (data not shown).

T production by primed incubates was elevated during hours 3–6 and 6–9 of incubation as compared to the initial interval (Fig. 4), and then decreased for the duration of the experiment. T production by unprimed incubates increased steadily throughout the experiment. Basal production of T in control cultures containing primed incubates was approximately 5-fold greater than basal production of T by unprimed incubates (data not shown).

Effects of steroids and inhibitors on FOM in vitro

The effects of trilostane on hCG-induced follicular DHP and 20β-S production and oocyte GVBD *in vitro* are shown in Figure 5. Significant decreases in media concentrations of DHP and 20β-S occurred at trilostane concentrations ≥ 0.1 μg ml^{-1}. Significant reductions in the percentage of oocytes completing GVBD coincided with the decrease in steroid concentrations. At a trilostane concentration of 1 μg ml^{-1} media levels of DHP and 20β-S were non-detectable and oocyte GVBD was abolished.

Cycloheximide significantly reduced hCG-induced DHP production at a concentration of 0.001 μg ml^{-1} while oocyte GVBD was signifi-

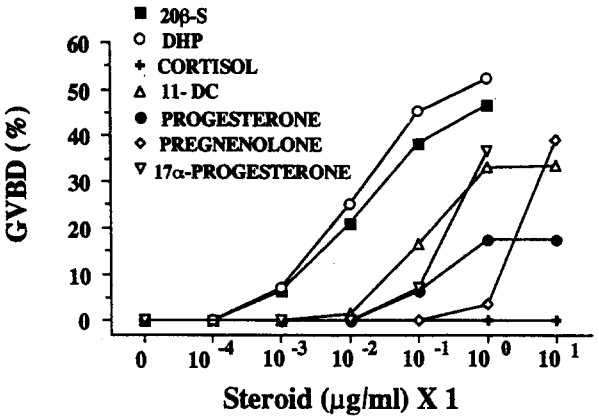

Fig. 7. Structure-activity relationships of steroids for their ability to induce oocyte germinal vesicle breakdown (GVBD) in white perch ovarian fragments (100 mg) *in vitro*. Ovarian fragments were incubated in the presence or absence of 6 concentrations of authentic steroids for up to 66 h in the bioassay. 20β-S, 17α,20β,21-trihydroxy-4-pregnen-3-one; DHP, 17α,20β-dihydroxy-4-pregnen-3-one; cortisol, 11β,17α,21-trihydroxy-4-pregnen-3-one; 11-DC (11-deoxycortisol), 17α,21-dihydroxy-4-pregnen-3-one. Symbols represent the mean value for n = 3 incubations.

cantly inhibited at cycloheximide concentrations ≥ 0.01 μg ml^{-1} (Fig. 6). DHP production was nearly non-detectable and oocyte GVBD was eliminated at cycloheximide concentrations $\geq$ 0.1 μg ml^{-1}. 20β-S levels in culture media were near or below detection limits for all concentrations of cycloheximide tested.

The relationships between steroid structure and their ability to induce GVBD in white perch oocytes is shown in Figure 7. DHP and 20β-S were equipotent in their ability to induce GVBD and were the only steroids tested that could induce GVBD within a physiological range of concentrations. The unsubstituted steroids, pregnenolone and progesterone, were relatively ineffective for inducing GVBD in the bioassay. The addition of a hydroxyl group at the 17α position (17α-progesterone) did not enhance the ability of the steroid to induce GVBD. Hydroxylation at the 17α and 21 positions (11-deoxycortisol; 11-DC) significantly improved GVBD-inducing activity. When 20β-hydroxylation was combined with either 17α hydroxylation (DHP) or hydroxylation at the 17α and 21 positions (20β-S), the GVBD-inducing potency increased significantly when compared to the other steroids tested.

Discussion

The results of the present study show that both immunoreactive DHP and 20β-S are present in the plasma of white perch and white bass during hCG-induced FOM. This finding corroborates a previous report on elevated levels of these two hormones in striped bass during natural and pharmacologically-induced FOM (King *et al.* 1994a). In that study, approximately half of the 20β-S immunoreactivity coeluted with a 5β,3α-hydroxylated form of 20β-S identified by reverse-phase HPLC of extracted plasma. The differences in relative levels of DHP and 20β-S in maturing perch and bass in the present study may also be due to synthesis of reduced metabolites of DHP or 20β-S that cross-react in varying degrees with the RIA antisera (see Hourigan *et al.* 1991; King *et al.* 1994a).

The results of experiments evaluating the time course of steroidogenesis during *in vitro* FOM indicate that a priming injection of hCG is necessary for white perch follicles to produce DHP and 20β-S and for their oocytes to complete GVBD. Ovarian fragments from primed fish produced DHP and 20β-S and their oocytes underwent GVBD when exposed to hCG *in vitro*. Ovarian fragments from perch receiving vehicle alone (unprimed) did not produce DHP or 20β-S when exposed to hCG *in vitro* and their oocytes did not mature. This priming injection may mimic an increase in circulating endogenous GTH (Nagahama 1987a) and induce production of factor(s) necessary for MIH action or MIH synthesis such as an oocyte MIH receptor (Patino and Thomas 1990c) or 20β-hydroxysteroid dehydrogenase (Nagahama 1987a).

In contrast to the results with perch, oocytes from unprimed white bass matured to an advanced stage (GVM) *in vitro* after exposure of ovarian fragments to hCG and their oocytes were competent to complete GVBD in response to DHP or 20β-S (data not shown). The white bass used in the present study were exposed to a rapid increase in water temperature just prior to the experiments. Abrupt increases in water temperature have been associated with the preovulatory GTH surge in goldfish, *Carassius auratus*, (Kobayashi *et al.* 1987, 1988) and are a commonly used method to induce

FOM and ovulation in this species (Yamamoto *et al.* 1966). Striped bass show an enhanced response to hCG or synthetic gonadotropin releasing-hormone analogues if they are exposed to an increase in water temperature just after ovarian growth is complete (C. Sullivan and R. Hodson, *unpublished data*). These findings suggest that the white bass oocytes may have been primed *in vivo* by endogenous GTH prior to being sampled for ovarian tissue. Further experiments are required to verify this interpretation.

In white bass and white perch, plasma levels of E_2 and T were greatest when their largest oocytes were undergoing GVM, reaching levels comparable to white perch sampled during their natural spawning season (Jackson and Sullivan 1995). Although plasma E_2 and T levels decreased in both white perch and white bass after their largest oocytes completed GVBD, they remained substantial in animals undergoing oocyte hydration and ovulation. These results are consistent with circulating levels of gonadal steroids reported for other multiple-clutch batch spawning species (Thomas *et al.* 1987; Pankhurst and Carragher 1992) including white perch sampled during natural FOM (Jackson and Sullivan 1995). Production of E_2 and T by ovarian incubates from fish primed with hCG decreased (white bass) or remained relatively unchanged (white perch) during *in vitro* FOM. In species containing ovarian follicles which can be easily separated by maturational stage, secondary (vitellogenic) follicles produced relatively high levels of E_2 when compared to post-vitellogenic follicles undergoing FOM (Kagawa *et al.* 1984). Taken together, these findings suggest that vitellogenic follicles of white perch and white bass produced E_2 and T in response to hCG while follicles undergoing FOM produced DHP and 20β-S.

Trilostane effectively blocked DHP and 20β-S production and associated oocyte GVBD in white perch follicles *in vitro* indicating that the GTH-induced production of the MIH in this species follows a $\Delta^5-\Delta^4$ pathway for steroid synthesis as in the congeneric striped bass (King *et al.* 1994b) and some other teleosts (Young *et al.* 1982; Patino and Thomas 1990b,c). In agreement with previous studies (Jalabert 1976; Patino and Thomas 1990c; King *et al.* 1994b), cycloheximide blocked DHP and 20β-S synthesis and oocyte GVBD in white perch follicles *in vitro* indicating that protein synthesis is required for FOM to proceed. These newly translated proteins may include MIH oocyte receptors (Patino and Thomas 1990c) or proteins necessary to activate a maturation promoting factor (Nagahama *et al.* 1993).

The preliminary evaluation of structure-activity relationships for GVBD-inducing steroids showed that, as in other teleosts, (Goetz 1983; Scott and Canario 1987) DHP and 20β-S were equally potent steroids and highly effective for inducing *in vitro* oocyte GVBD in white perch follicles. We now have conclusive evidence that the congeneric striped bass possesses an ovarian membrane receptor for 20β-S but lacks a comparable DHP receptor (King 1995). The bioactivity of DHP and the other C_{21} steroids tested may be due to their conversion to 20β-S. Atlantic croaker ovarian fragments are known to convert DHP and 11-DC to 20β-S *in vitro* (Patino and Thomas 1990a). In addition, only a 1 min exposure to 20β-S is necessary for oocyte GVBD to occur while the GVBD-inducing effectiveness of DHP is diminished with decreasing exposure time (Ghosh and Thomas 1993). In the present study, incubation times often exceeded 30 h and GVBD was used as an endpoint, so exogenous steroids had ample time to be converted to active products. In ongoing analyses we will determine the conversion rates of labeled steroid precursors by *Morone* ovarian fragments over various time intervals. In agreement with structure-activity studies in Atlantic croaker (Trant and Thomas 1988) and striped bass (King *et al.* 1994b) cortisol (oxygenated at position 11) was impotent regardless of the concentration tested (up to 10 $\mu g\ ml^{-1}$).

In summary, changes in circulating steroid hormone levels and ovarian steroidogenesis during *in vivo* and *in vitro* FOM were investigated in white perch and white bass. Increases in plasma immunoreactive DHP and 20β-S were associated with specific, progressive stages of oocyte maturation in white bass undergoing hCG-induced FOM. Significant increases in immunoreactive 20β-S were not detected in the plasma of white perch undergoing hCG-induced FOM. Levels of DHP increased with

oocyte GVM in cultures of ovarian fragments from females primed with hCG. While 20β-S was detectable in cultures of the primed incubates, it was always produced at much lower levels than DHP. Ovarian incubates from unprimed perch and bass produced only E_2 and T and their oocytes did not complete GVBD; however, unprimed white bass oocytes initiated FOM indicating their potential as a model for the development of maturational competence. These results provide preliminary evidence for the involvement of both DHP and 20β-S in the regulation of FOM in these species.

Acknowledgements

We thank Mr. L. Brothers of Carolina Fisheries for providing the white bass used in this study and Dr. R.G. Hodson for assistance with establishing and managing the perch broodstock. We are grateful to Dr. Y. Nagahama for providing the T and DHP antisera and to Drs. P. Thomas and G. Niswender for supplying 20β-S and E_2 antisera, respectively. This work was supported by grants from the University of North Carolina Sea Grant College Program (#NA86AA-D-SG062 and #NA90AA-D-S6062) and the National Coastal Resources Researches and Development Institute (#NA87AA-D-S6065, contract #2-5606-22-2).

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