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The *in Vitro* Effects of Various Steroid Hormones and Gonadotropin on Oocyte Maturation of the Viviparous Rockfish, *Sebastes taczanowskii*

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Abstract

The effects of seven steroid hormones (progesterone, 17α-hydroxyprogesterone, 17α, 20β-dihydroxy-4-pregnen-3-one, deoxycorticosterone, cortisol, testosterone and estradiol-17β) and human chorionic gonadotropin on the induction of *in vitro* oocyte maturation were examined in a viviparous rockfish, *Sebastes taczanowskii*. Among them, 17α, 20β-dihydroxy-4-pregnen-3-one was the most potent steroid in inducing *in vitro* germinal vesicle breakdown (GVBD), a criterion for oocyte maturation in follicle-enclosed oocytes. Progesterone, 17α-hydroxyprogesterone and deoxycorticosterone were moderately effective only at a high dose, while cortisol was slightly effective. Testosterone and estradiol-17β did not have any effect at the concentrations tested. Human chorionic gonadotropin was very effective with rates of GVBD similar to those of 17α, 20β-dihydroxy-4-pregnen-3-one. Moreover, human chorionic gonadotropin was effective in stimulating 17α, 20β-dihydroxy-4-pregnen-3-one synthesis by follicle-enclosed oocytes. These results suggest that 17α, 20β-dihydroxy-4-pregnen-3-one is the maturation inducing steroid of this species and that synthesis of the steroid is regulated by gonadotropin.

Introduction

Oocyte development and maturation in teleosts are regulated directly and indirectly by various hormones in the hypothalamus-pituitary-ovary axis. For the majority of fish species studied, oocyte maturation including germinal vesicle breakdown (GVBD) is particularly induced by either 11-deoxycorticoids or progestogens (cf. Goetz, 1983). Many studies have demonstrated that in various teleosts, 17α, 20β-dihydroxy-4-pregnen-3-one (17α, 20β-diOHprog) is one of the most potent progestogens in inducing oocyte maturation, based on *in vitro* experiments (Duffey and Goetz, 1980; Nagahama et al., 1980; Young et al., 1982; Nagahama et al., 1983; Yamauchi et al., 1983; Upadhyaya and Haider, 1986; Adachi et al., 1988) and serum profiles of the steroid during the maturation period (Kagawa et al., 1983; Young et al., 1983; Ueda et al., 1984; Yamauchi et al., 1984; Shimizu et al., 1985; Levavi-Zermonsky and Yaron, 1986).

In viviparous teleosts, although a few studies on hormonal regulation of gestation and/or parturition have been carried out (Ishii, 1962 and 1963; Korsgaard and Petersen, 1979; deVlaming et al., 1983; Groves and Batten, 1986), there have been no reports on regulation of oocyte maturation. Fertilization, gestation and

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parturition are a chain of events which follow on oocyte maturation in the ovary of viviparous species. To clarify these peculiar events associated with viviparity, it is very interesting to investigate the endocrine mechanisms which regulate oocyte maturation. The present study was designed to examine the effect of various hormones on oocyte maturation of a marine viviparous rockfish, *Sebastes taczanowskii*, by an *in vitro* incubation method. An experiment on *in vitro* 17α, 20β-diOHprog synthesis in the ovarian follicles by addition of gonadotropin was also carried out.

Material and Methods

The white-edged rockfish, *Sebastes taczanowskii*, used in this study were collected by angling or with gill nets along the shores of Shikabe and Esan in the suburbs of Hakodate, southern Hokkaido from spring to fall in 1986. These were reared in indoor circular tanks (1 ton capacity) with seawater flowing through under natural water temperature and daylight condition until use in late March to early April of 1987. After anesthetization in ethyl 4-aminobenzoate, ovaries were dissected out and placed in cold Eagle's MEM solution buffered by 10 mM Hepes (pH 7.6). Follicle-enclosed oocytes were then teased from the overlaying connective tissues.

Effects on GVBD in oocytes were evaluated by seven steroid hormones: testosterone (T) and estradiol-17β (E_2), 17α, 20β-diOHprog, 17α-hydroxy-progesterone (17α-OHprog), progesterone (Prog), deoxycorticosterone (DOC), cortisol (F) (Sigma, USA). Stock solutions of each steroid were dissolved in ethanol and then diluted with Eagle's MEM to give final concentrations of 0.001, 0.01, 0.1 and 1.0 μg/ml which contained less than 1% ethanol. In addition, the effect of human chorionic gonadotropin (HCG; Teikoku zohki, Tokyo, Japan) on the induction of GVBD was examined. HCG (1, 10, 100 IU/ml) was directly dissolved in Eagle's MEM. Fifty oocytes were incubated in each hole of Nunclon (Nunc, Denmark) with or without hormones for 72 hours at 10°C. Three replicates were conducted for each treatment.

To determine *in vitro* 17α, 20β-diOHprog synthesis, 30 oocytes were incubated in three HCG concentrations (1, 10 and 100 IU/ml) for 72 hours at 10°C. After incubation, oocytes with induced GVBD were counted and the incubation media were kept at −40°C until analysis. The steroid was measured using the radioimmunoassay method of Kagawa et al. (1981).

All data are expressed as mean±SEM. Duncan's multiple range test was used for statistical analysis.

Results and Discussion

As oocyte maturation progressed, the germinal vesicle became visible as it migrated from the inner portion of the cell to the animal pole (Fig. 1a). Subsequently, the germinal vesicle disappeared (GVBD) and the oocyte became translucent at the same time (Fig. 1b). During the course of maturation, small oil droplets dispersed through the ooplasm, and then gradually aggregated to form a large, prominent oil globule. Oocytes also increased significantly ($p<0.01$) in diameter during this process (Table 1). In this study, GVBD was used as a criterion for determining final maturation.

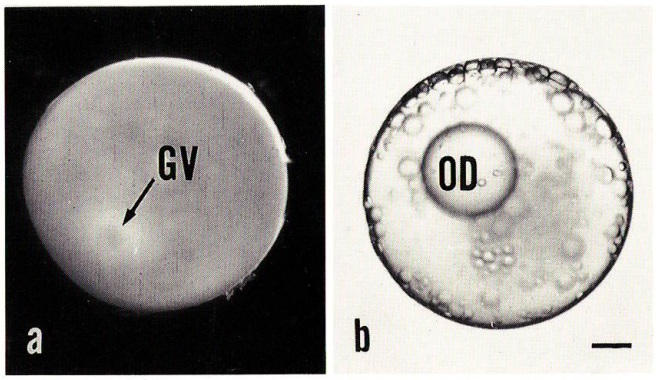

Fig. 1. Changes prior to (a) and after (b) germinal vesicle breakdown in *S. taczanowskii* oocytes. The bar represents 100 μm. GV and OD show a germinal vesicle and oil droplet, respectively.

Table 1. Changes of oocyte diameter prior to and after oocyte maturation

	maturing	matured
Egg diameter (mm)	0.799±0.003 (23)[a]	0.816±0.004*(23)

[a] Number of oocytes measured. Each value represents the mean± standard error.

* Significantly different from maturing oocytes at $p<0.01$.

The effects of the seven steroid hormones (T, E_2, DOC, F, Prog, 17α-OHprog and 17α, 20β-diOHprog) tested for *in vitro* induction of GVBD in isolated oocytes are shown in Fig. 2. Among these hormones, only T and E_2 did not have any effect at the concentrations tested.

DOC was moderately effective at 1.0 μg/ml (53% GVBD), while F was slightly effective even at the same concentration (10% GVBD). In several species, 11-corticosteroids such as DOC are as potent as progestogens in inducing oocyte maturation (Nagahama et al., 1980; Duffey and Goetz, 1980; Young et al., 1982; Yamauchi et al., 1984; Upadhyaya and Haider, 1986; Adachi et al., 1988). A positive interpretation of the effect of DOC must be deferred, because physiological information such as the serum profile and gonadal synthesis of corticosteroids have not been obtained in *S. taczanowskii*.

Of the three progestogens used in this study, Prog and 17α-OHprog, as well as DOC, were moderately effective only at a high dose (1.0 μg), showing GVBD rates of 61% and 68%, respectively. The most effective steroid was 17α, 20β-diOHprog which induced GVBD in 82% of oocytes tested at a concentration of 1.0 μg/ml. At a level of 0.1 μg/ml, this inducer also produced a 75% response, although the other six steroids were ineffective or much less effective at the same level. It has been reported that 17α, 20β-diOHprog has the most potent effect in inducing *in vitro* oocyte maturation in fish (Duffey and Goetz, 1980; Nagahama et al., 1980; Young et al., 1982; Nagahama et al., 1983; Yamauchi et al., 1983; Upadhyaya and Haider, 1986; Adachi et al., 1988). In addition, the levels of this hormone in the blood reached a peak near the time of oocyte maturation in salmonids (Young et al.,

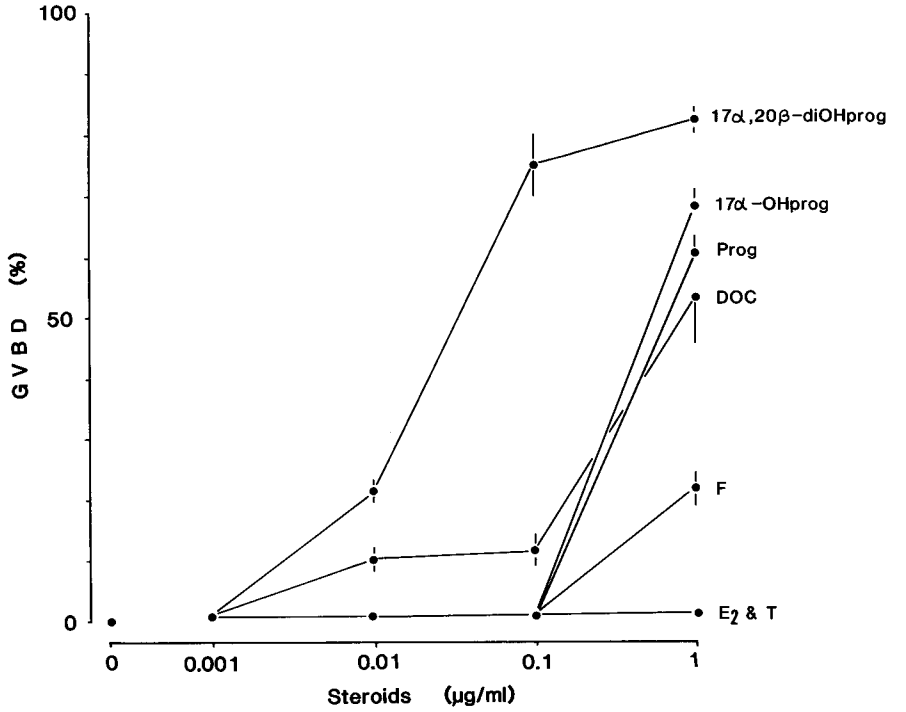

Fig. 2. *In vitro* effects of various steroid hormones on inducing germinal vesicle breakdown in *S. taczanowskii* oocytes.

1983 ; Yamauchi et al., 1984 ; Ueda et al., 1984), goldfish (Kagawa et al., 1983), carp (Levavi-Zermonsky and Yaron, 1986) and bitterling (Shimizu et al., 1985). The results described above show that 17α, 20β-diOHprog is the maturation inducing steroid in many oviparous species. Based on the results of this study, it is suggested that 17α, 20β-diOHprog is also the maturation inducing steroid in *S. taczanowskii*.

The present study also showed that HCG was considerably effective at 1, 10 and 100 IU/ml with GVBD of 11%, 77% and 86%, respectively (Fig. 3). Although HCG has been used extensively for the *in vivo* induction of final maturation and ovulation, it is less active *in vitro* in many fish (Goetz, 1983). Among those species examined, HCG has been shown to be very effective in inducing *in vitro* GVBD in killifish, *Fundulus heteroclitus* (Wallace and Selman, 1980) as well as in *S. taczanowskii*. The causes of these differences in biological activity of HCG among different fishes are still unknown. In a preliminary experiment, it has been ascertained that the pituitary preparation of *S. taczanowskii* was also very effective, inducing 91% and 52% responses at levels of 1% and 0.1% (w/v) fresh pituitary suspension, respectively (Takemura, unpublished). These facts suggest that the pituitary and gonadotropin preparations are also effective in inducing *in vitro* oocyte maturation in this fish.

Furthermore, the effect of HCG on *in vitro* 17α, 20β-diOHprog synthesis by follicle-enclosed oocytes of *S. taczanowskii* was examined in this study. As shown in Fig. 4, HCG was significantly ($p<0.01$) effective in stimulating 17α, 20β-diOH-

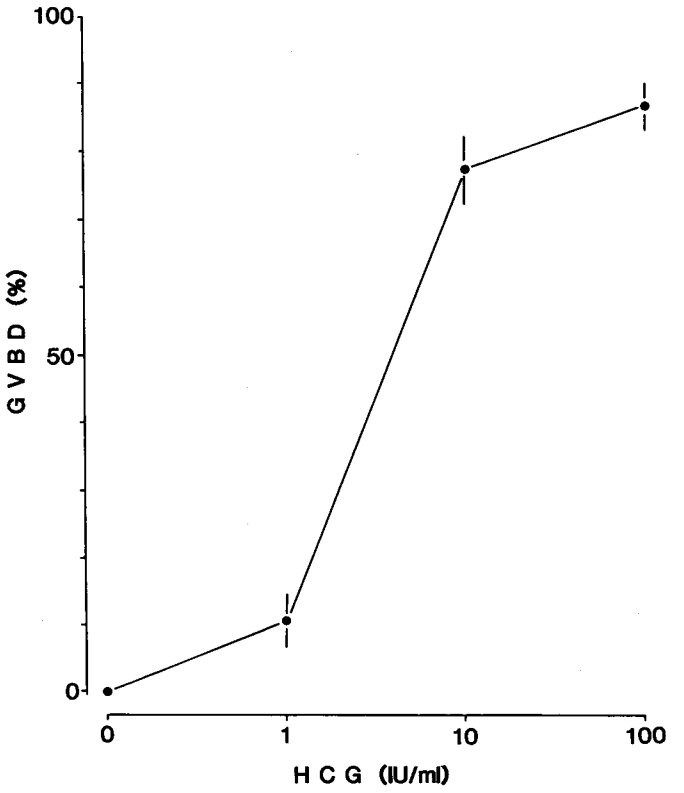

Fig. 3. An *in vitro* effect of HCG on inducing germinal vesicle breakdown in *S. taczanowskii* oocytes.

prog synthesis at concentrations of 10 and 100 IU/ml. In some fish species, the action of gonadotropin in inducing oocyte maturation appears to be dependent on the synthesis of a secondary steroidal effector, a maturation-inducing hormone (Goetz, 1983; Nagahama, 1987). Nevertheless definite evidence for the action of HCG is lacking in this study; the results obtained suggest that the pathway leading to the induction of final maturation in viviparous scorpaenids is similar to that in oviparous species already investigated (Goetz, 1983).

Groves and Batten (1986) suggested that in a viviparous teleost, *Poecilia latipinna*, 17α, 20β-diOHprog influenced the female reproductive cycle at ovulation/parturition rather than at oocyte maturation at the hypophyseal level, based on ultrastructural observations of *in vitro* effects of some gonadal steroids on the gonadotrophs. During the reproductive cycles of *S. taczanowskii* and a related species, *S. schlegeli*, however, the serum concentrations of 17α, 20β-diOHprog have been observed to increase around the estimated time of final oocyte maturation and subsequent gestation (Takemura, unpublished). Therefore, it is likely that 17α, 20β-diOHprog is at least partially involved in oocyte maturation and gestation maintenance in viviparous scorpaenids.

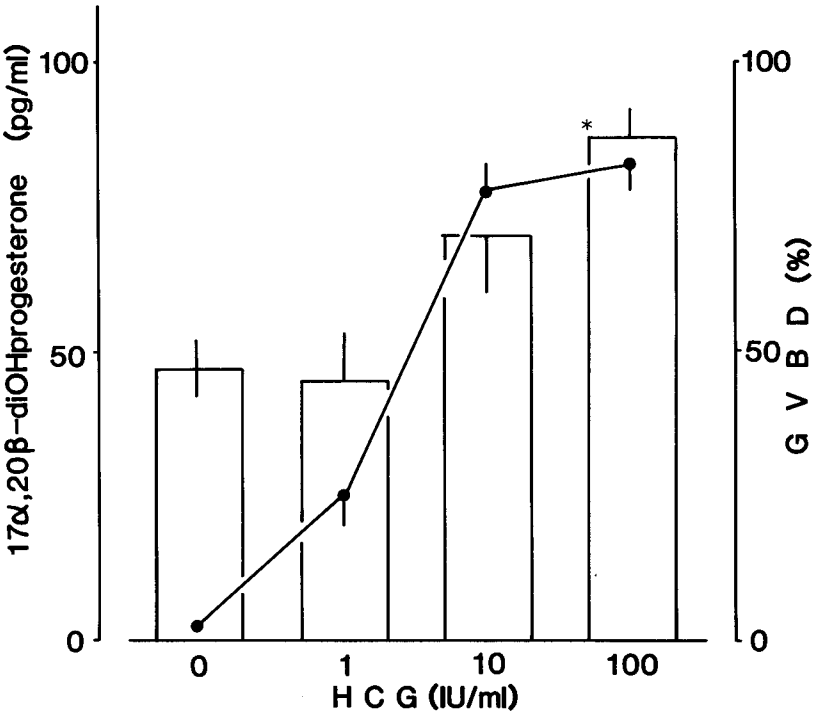

Fig. 4. *In vitro* synthesis of 17α, 20β-diOHprog (bar graph) and the percentages of oocytes having induced germinal vesicle breakdown (line graph) at various HCG concentrations. * Indicates significant difference ($p<0.01$) compared to control.

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