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Biological Study on Ovulation *in vitro* of Fish—VI Effects of Metopirone (SU-4885) on Salmon Gonadotropin- and Cortisol-induced *in vitro* Ovulation in *Oryzias latipes**

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This investigation was carried out to asertain whether corticosteroidogenesis in the follicular tissues is required for gonadotropin-induced ovulation of the *Oryzias* oocyte. A specific inhibitor (SU-4885) of 11β-hydroxylase resulted in inhibition of gonadotropin-induced ovulation but did not affect ovulation induced by cortisol. Furthermore, it was shown that prior exposure of oocytes to metopirone reduced the response of the oocytes to gonadotropin but ovulation induced by cortisol was not affected by the prior treatment. These results suggest that an inhibition of corticosteroidogenesis prevents ovulation and prior treatment 2 hours in advance is the period necessary for corticosteroidogenesis in the follicular tissues in *Oryzias latipes*.

It has been shown[1–3] that mammalian and fish gonadotropins are very effective in inducing ovulation *in vitro* in *Oryzias latipes* when the full grown oocytes are used. In *Oryzias*[4] as well as the catfish,[5–7] cortisol also induced ovulation *in vitro*. Furthermore, as has been previously suggested with regard to ovulation in *Oryzias latipes*[4], ovulation is induced by activating both the pituitary-interrenal-ovary and the pituitary-ovary systems. In contrast, only the pituitary-interrenal-ovary system is involved in ovulation in the catfish. Luteinizing hormone (LH) does not act directly on the ovary in the catfish, but rather via the interrenal gland.

Recently, HIROSE[8] has indicated that ovulation can not be induced in *Oryzias* oocytes dissected from ovarian follicles prior to exposure to hormone, suggesting that gonadotropin acts first on the follicular tissues to produce effective agents which in turn are responsible for ovulation in the fish. The follicular tissues appear to be the main site of hormonal action during maturation and ovulation in the fish. Furthermore, the follicular tissues comprised of granulosa and theca cells in teleosts are proposed to have the ability to secrete steroid hormones using histochemical techniques,[9–12] but the key enzymes (e.g., 11β-hydroxylase) involved in corticoid biosynthesis are not histochemically demonstrable in the follicles. However, 17-ketosteroids are found in the ovary of *Salmo salar*[13]. 11-ketotestosterone is also demonstrable in the ovaries of some teleosts, indicating the presence of 11β-hydroxylase[14–16]. Thus, although gonadotropin may induce ovulation by stimulating interrenal corticosteroidogenesis, gonadotropin may also induce ovulation

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by activating the processes involved in corticosteroidogenesis within the follicular tissues in *Oryzias latipes*.

Metopirone (SU–4885) is a specific inhibitor of 11β-hydroxylase at low levels in the adrenal glands of mammals[17,18] and is used to study pituitary-interrenal function in some teleosts[19–26] as well as higher vertebrates. This drug at appropriate dosage appears to be exerting a marked inhibitory effect on 11β-hydroxylation in some teleosts[25]. Metoporione may be, therefore, useful in the elucidation of the presence of key enzymes related to corticosteroidogenesis in the teleost ovary. This report describes the effects of metopirone on gonadotropin- and cortisol-induced ovulation *in vitro* of *Oryzias latipes* oocytes. Special attention is given to the possibility that corticosteroidogenesis in the follicular tissues is necessary for ovulation.

Materials and Methods

The red variety of *Oryzias latipes* were maintained under conditions similar to those described in the previous report.[1] Ovaries were removed from the fish and placed in a sterlized medium 199. The individual oocytes were manually removed from the ovary with dissecting pins. Each contained one oocyte surrounded by the follicular tissues. In each experimental group (see Tables 1 and 2), 9 to 14 individual oocytes were immersed in 3 m*l* of medium on a watch glass covered with a Petri dish, and similar trials were replicated. In this study, two experiments were conducted. In experiment 1, metopirone, SU–4885 (CIBA, 2-methyl-2 bis(3-pyridyl)-1-propanone) was added simulatenously with salmon pituitary gonadotropin (SG–G100) or cortisol (Sigma, hydrocortisone acetate). In experiment 2, after prior exposure of *Oryzias* oocytes to metopirone for 2 hours, the oocytes were washed twice in a fresh medium and then immersed in a fresh medium containing hormone.

The partially purified salmon piutitary gonadotropin[27] was used at a concentration of 4 μg/m*l*. Cortisol was dissolved in ethanol and propylene glycol (1: 1), and its concentration was 1 μg/m*l*. The concentrations of both hormones were chosen based on the results of previous investigations.[3,4] Metopirone was also dissolved in ethanol and propylene glycol (1: 1) and used at concentrations of 1 and 10 μg/m*l*. 0.01 ml of cortisol or metopirone solution was added to 3 m*l* of the medium.

Two experiments were initiated at 5.00 p.m., and at 10.00 a.m. on the following day the oocytes were observed under a dissecting microscope. The percentage which had ovulated was determined by observation of germinal vesicle breakdown, and also the presence of villi and attached filaments around the ovulated eggs as a consequence of removal of the follicular tissues.

Results

In experiment 1, the oocytes received no previous treatment of metopirone. Metopirone shows a clear inhibition of gonadotropin-induced ovulation when metopirone at a concentration of 1 or 10 μg/m*l* is added simulatenously with salmon gonadotropin at 5.00 p.m. (Table 1). The percentage of ovulation is clearly less in the gonadotropin and metopirone-treated group than that in the gonadotropin-treated one. Difference in the values between both groups is statistically significant, $p<0.01$. Cortisol-induced ovulation is, however, unaffected by addition of metopirone, group 7 (68.8 ± 4.3) which received metopirone at 1 μg and cortisol shows almost similar percentage of ovulation as group 3 (70.8 ± 4.1) which received only cortisol. Moreover, ovulation induced by cortisol is not inhibited by the addition of a higher dose of metopirone at 10 μg/m*l* (group 6, Table 1).

Table 1. Effect of metopirone on *in vitro* ovulation of *Oryzias* oocytes.

Group No.	Treatment	No. of experiment	Percentage of ovulation (mean±standard error)
1	control	3	0
2	gonadotropin 4 μg/m*l*	3	64.1 ± 2.6
3	cortisol 1 μg/m*l*	3	70.8 ± 4.1
4	metopirone 10 μg/m*l* + gonadotropin 4 μg/m*l*	3	11.6 ± 5.8*
5	metopirone 1 μg/m*l* + gonadotropin 4 μg/m*l*	3	36.1 ± 2.9*
6	metopirone 10 μg/m*l* + cortisol 1 μg/m*l*	3	54.5 ± 6.9**
7	metopirone 1 μg/m*l* + cortisol 1 μg/m*l*	3	68.8 ± 4.3**

* Significant compared with group 2 ($p<0.01$).
** Not significant compared with group 3.

Table 2. Effect of prior exposure of metopirone on *in vitro* ovulation of *Oryzias* oocytes.

Group No.	Pre-treatment	Treatment	No. of experiment	Percentage of ovulation (mean±standard error)
1	metopirone 1 μg/m*l*	gonadotropin 4 μg/m*l*	3	32.9 ± 4.5
2	metopirone 10 μg/m*l*	gonadotropin 4 μg/m*l*	3	13.5 ± 4.3
3	metopirone 1 μg/m*l*	cortisol 1 μg/m*l*	4	53.9 ± 2.2*

* Significant compared with group 1 ($p<0.05$).

It has been previously shown[28] that exposure of *Oryzias* oocytes to gonadotropin or cortisol for a 2 hour period (5.00–7.00 p.m.) is sufficient to induce ovulation *in vitro*. Therefore, after the prior exposure to metopirone for 2 hours, the oocytes are washed and incubated in the fresh medium containing salmon gonadotropin or cortisol (Table 2).

It is clearly shown that the prior exposure to metopirone induces a much reduced response of the *Oryzias* oocyte to gonadotropin, but ovulation induced by cortisol is not affected by the prior treatment with the inhibitor. The percentage of ovulation is significantly greater in group 3 than that in group 1 ($p<0.05$ as shown in Table 2).

Discussion

It has never been reported whether or not 11β-hydroxylase, related to corticoid biosynthesis, is present in the follicular tissues of oocytes in teleost. It is, however, shown that ovaries in some teleosts are capable to synthesize 11-ketotestosterone, which probably indicates the presence of 11β-hydroxylase. Moreover, 17-ketosteroids,[29] which are known to be metabolites of cortisol and deoxycorticosterone, are extractable from the ovary of *Salmo salar*.[13] In the sychronous hermaphrodite teleost (*Serranus scriba*),[30] corticoids have been demonstrated in the gonad.

Recently, COLOMBO *et al.** have shown that teleost ovaries can synthesize 11-deoxycorticoids which may act locally to induce maturation and ovulation of oocytes. HIROSE *et al.* (unpublished) similarly indicate production of deoxycorticoids accompanied by 21-hydroxylase when the follicular tissues of trout oocytes with or without gonadotropin are incubated. Therefore, metopirone appears to abolish the response of *Oryzias* oocytes to gonadotropin, while ovulation by cortisol shows no effect after the addition of metopirone. The above discussion sugguests that the follicular tissues in *Oryzias* oocyte are related to corticosteroidogenesis with 11β-hydroxylase and 21-hydroxylase and that gonadotropin may activates the enzymatic pathways of corticoids in the follicles especially during maturation and ovulation in *Oryzias latipes*.

Indian scientists[6,7] suggest that deoxycorticosterone and cortisol have their action directly on the catfish oocyte for inducing ovulation. In contrast to the catfish, deoxycorticosterone is not so clearly effective in inducing ovulation *in vitro* of *Oryzias* oocytes (unpublished), while cortisol is very effective in inducing ovulation.[4] However, cortisol can not induce *in vitro* ovulation of *Oryzias* oocytes removed from their follicular tissues.[8] This suggests that cortisol is not required directly for the event of ovulation and an effective secondary substance, which may be produced in the processes of corticoid biosynthesis or metabolism in the follicular envelope, seems to be directly responsible for ovulation *in vitro* of *Oryzias* oocytes. Therefore, ovulation which is instigated by the pituitary-ovary system in *Oryzias latipes*, may be induced by some sort of an effective secondary substance which is produced during processes of corticoid biosynthesis or metabolism within the follicular envelope when such is activated by addition of gonadotropin.

In experiment 2, *Oryzias* oocytes are exposed to metopirone for 2 hours (5.00–7.00

* L. COLOMBO, H. A. BERN, J. PIEPRZYK, and D. W. JOHNSON: *Gen. Comp. Endocrinol.*, in press (1973).

p.m.), which are sufficient to induce ovulation *in vitro*[28], prior to the continuous treatment of hormone. In this case, response of the oocytes to gonadotropin is reduced in a similar fashion to the result shown in Table 1. Therefore, this result indicates that the prior treatment 2 hours may be the period necessary for corticosteroidogenesis in the follicular tissues in *Oryzias latipes* oocyte.

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