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Regulation of oocyte maturation in the rainbow trout, *Salmo gairdneri*: role of cyclic AMP in the mechanism of action of the maturation inducing steroid (MIS), 17 α -hydroxy, 20 β -dihydroprogesterone

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Abstract

In fish, oocyte maturation (resumption of meiosis after completion of vitellogenesis and before ovulation) is triggered by maturation inducing steroids (MIS) which generally appear to be secreted in the ovary in response to stimulation by a pituitary maturational gonadotropin. Converging data from different laboratories show that 17 α -hydroxy, 20 β -dihydroprogesterone (17 α , 20 β -OH-P) is the principal MIS in salmonoids; but clear identification remains to be done in other taxonomic groups.

The experiments reported here in the rainbow trout *Salmo gairdneri* examine the possible involvement of oocyte cAMP on the mechanism of MIS action. The action of 17 α , 20 β -OH-P, on germinal vesicle breakdown (GVBD) in oocytes incubated *in vitro* within the follicle, was inhibited by various substances expected to elevate the intraoocyte concentrations of cAMP: cAMP (≥ 1 mM) or dibutyl cAMP (≥ 2 mM), phosphodiesterase inhibitors such as theophylline (≥ 0.2 mM) or 3-isobutyl-1 methylxanthine (IBMX ≥ 0.1 mM), adenylate cyclase activators such as cholera toxin (> 100 nM) or forskolin (≥ 0.03 mM). In fact, the combined action of IBMX (1 mM) and forskolin (0.01 or 0.05 mM) *in vitro* was to promote accumulation of intraoocyte cAMP within 1 to 5 hours. Oocyte cAMP concentrations exhibited a large variability between different females, depending on the stage of oocyte development; a significant positive correlation between oocyte cAMP concentration and the follicular weight, and a significant negative correlation between oocyte cAMP concentration and the median efficient dose of 17 α , 20 β -OH-P for induction of GVBD, were observed. Finally, when intrafollicular oocytes were incubated *in vitro*, the addition of a maturation-inducing concentration of 17 α , 20 β -OH-P (3×10^{-6} M) induced a significant decrease of oocyte cAMP within the first 10 hours of incubation. These results show that cAMP appears to play a central role in the regulation of oocyte sensitivity to 17 α , 20 β -OH-P and in the intraoocyte mechanisms leading to GVBD in trout.

These data are discussed together with the few indications available in fish concerning the mechanism of MIS action which can be compared to some extent with the amphibian model.

Introduction

Spawning of female oviparous fish is preceded by oocyte (meiotic) maturation and ovulation. The term oocyte maturation refers here to a short phase of oogenesis characterized by the resumption of

meiosis which occurs after the vitellogenic growth during which meiosis had been arrested in prophase of first division, up to a further arrest usually in metaphase of second division in most vertebrates.

Although oocyte maturation can be accompanied by obvious morphological changes in some

fish species, such as large swelling due to hydration, and (or) cytoplasm clearing due to the coalescence of lipid droplets and yolk globules, the most universal and reliable criterion is the disappearance of the germinal vesicle, called germinal vesicle breakdown (GVBD) (see Goetz, 1983, for review).

It is now well documented in various fish species that oocyte maturation is controlled by maturation inducing steroids (MIS) produced by the follicular envelope, in response to an hypophysial maturational gonadotropin (Fostier and Jalabert, 1982; Goetz, 1983). From converging experimental evidence, 17α -hydroxy, 20β -dihydroprogesterone (4 pregnen- 17α , 20β diol-3-one (17α , 20β -OH-P)) was first hypothesized to be the principal MIS in rainbow trout (Jalabert 1976). Further studies demonstrated that $17\alpha,20\beta$ -OH-P is present in large amounts in the plasma of this species around the time of maturation and ovulation (Campbell *et al.* 1980, Fostier *et al.* 1981a) and that it can be secreted *in vivo* by ovarian follicles in response to gonadotropin stimulation (Fostier *et al.* 1981b). Taking into account recent works carried out in other salmonoids (Nagahama *et al.* 1983, Nagahama and Adachi 1985) it can be assumed that $17\alpha,20\beta$ -OH-P is the principal MIS in salmonoids. However, the identification in the ovary of a salmonoid, the ayu (*Plecoglossus altivelis*), by Suzuki *et al.* (1981) of a 5β -reductase activity on $17\alpha,20\beta$ -OH-P and of a maturation inducing activity of the 5β -reduced metabolites, raised the question of which of these steroids can be considered as the MIS. Data presented here show that they are less effective than $17\alpha,20\beta$ -OH-P upon trout oocyte maturation.

Concerning the mechanism of MIS action on the oocyte, few data are presently available in fish, whereas numerous works have been performed in amphibians during recent years, mainly in *Xenopus*. The reasons for the considerable interest paid to the mechanism of oocyte maturation in *Xenopus* lie in particular qualities of the biological material (their availability, easy denudation of oocytes for *in vitro* culture, suitability for micro-injection of drugs within the ooplasm, etc.) which in turn offers a convenient example of steroid hormone target cell, which responds with a totally posttranscriptional mechanism, in contrast to

transcriptional mechanisms generally induced by steroids in other target cell systems. Although many points remain still obscure, or at least controversial, a general agreement seems now to exist concerning some principal steps in the mechanism of MIS action in *Xenopus* oocyte (see reviews by Maller and Krebs 1980; Maller 1985): progesterone (considered as the MIS in *Xenopus*) acts at the membrane level to temporarily inactivate membrane-bound adenylate cyclase, resulting in a transient decrease in intraoocyte level of cAMP. This in turn brings about a partial inactivation of a cAMP-dependent protein kinase which was maintaining initiator proteins in a phosphorylated inactive form. Activation of these initiator protein would then, *via* unknown steps involving protein synthesis to some extent (inhibition by cycloheximide), induce activation of a cytoplasmic maturation promoting factor (MPF) which is autocatalytically amplified.

In fish, the scant data prompt us to think that the main mechanisms could be similar: *in vitro* oocyte maturation induced by appropriate steroids can be blocked by protein synthesis inhibitors such as cycloheximide and (or) puromycin but not by transcription inhibitors (actinomycin D, mitomycin C), indicating that the mechanisms probably requires some protein synthesis but involves only posttranscriptional events (Detlaff and Skobolina, 1969; Goswami and Sundararaj, 1973; Jalabert, 1976). Observations by Voogt and De Groot (1983) on the stimulating effect of asterosaponins (steroidal saponins found in sea stars) on GVBD in zebrafish oocytes led them to hypothesize an irreversible action of these substances at the membrane level, thus demonstrating its implication in the first step of maturation initiation. Furthermore, the non-specific action of asterosaponins was observed to be followed by a significant decrease in oocyte cAMP. Concerning the possible implication of cAMP, indirect arguments come from the observations that phosphodiesterase inhibitors block $17\alpha,20\beta$ -OH-P induced oocyte maturation in brook trout (*Salvelinus fontinalis*) (Goetz and Hennessy, 1984). Finally the occurrence of MPF was reported in the sturgeon by Dettlaff 1977, cited by Masui and Clarke (1979).

Considering the amphibian 'model' of intracellular mechanisms where cAMP plays a central role, most of the works presented here were designed in order to determine whether cAMP could also be involved in a similar way in the mechanism of oocyte maturation in a salmonoid fish, the rainbow trout, *Salmo gairdneri*.

Material and methods

Fish

Female rainbow trouts (*Salmo gairdneri*), 2 and 3 years old, were used. They were acclimated in recirculated water units and checked once weekly to determine individual mean oocyte stage as already described (Jalabert and Fostier 1984a): end of vitellogenesis (EV), subperipheral germinal vesicle (SP-GV), peripheral germinal vesicle (P-GV).

Preparation of follicles for in vitro incubations

Fish exhibiting oocytes at the appropriate stage (SP-GV or P-GV) were sacrificed and the ovaries cooled and dissected at 4°C in a dissection medium (DM8/300) adjusted to pH 8 and osmotic pressure 300 mosmols (slightly modified from trout balanced salt solution (TBSS) (Jalabert and Fostier 1984b) and prepared as follows: 7.77g NaCl, 0.23 g KCl, 0.07 g Mg SO₄ · 7H₂O, 0.02g MG C12. 6H₂O, 0.5g Ca Cl₂ · 2H₂O and 0.48 g Hepes (Sigma: H-3375) dissolved in 800 ml of distilled water, pH adjusted to 8 using 0.04 M NaOH; the osmotic pressure was then adjusted to 300 mosmols by addition of water).

Isolated follicles, or clusters of 2 to 6 follicles (depending on the experiment) were thoroughly rinsed with DM8/300 to discard traces of blood and yolk, and kept at 4°C in DM8/300 before repartition in incubation vials.

Incubations of intrafollicular oocytes

Preincubation (without hormones) and incubations

were performed at 12.5°C under an atmosphere containing 50% N₂-50% O₂ in an incubation medium (IM8/300) pH 8, osmotic pressure 300 mosmols, prepared as for DM8/300 but with an increased amount of Hepes (4.77 g/l) and addition of glucose (1 g/l). Different kinds of vials were used, depending on the number of follicles required for the experiments, in order to provide a ratio of 5 to 6 follicles/ml, and allow them to lie in a single layer immersed in medium with a maximum exchange surface with atmospheric O₂. All vials were submitted to weak and continuous agitation during incubation.

Estimates of the median efficient dose (MED) of 17 α , 20 β -OH-P for oocyte maturation were performed as already described by Jalabert and Fostier (1984a).

For determination of the effective concentration of substances expected to inhibit oocyte maturation, follicles were first preincubated for 2h in various concentrations of these substances dissolved in IM8/300. 17 α , 20 β -OH-P was then added to the medium at a concentration (3×10^{-6} M) usually known to induce 100% GVBD even in oocytes at stage EV with a low sensitivity. The incubation of follicles in the presence of 17 α , 20 β -OH-P and inhibitors was carried out for 48h before observation of GVBD.

Oocyte extracts for cAMP assay

Unless otherwise mentioned, the following operations were performed between 0 and 4°C. Groups of 5 or 10 oocytes within follicular envelopes were weighed rapidly, placed in 1 ml syringes and squashed by forcing them through the small opening (diameter 1.5 mm) into 10 ml glass tubes containing 5 ml of squash medium (SM 7.5/300) prepared as for DM8/300 but buffered with Tris (50 mM) and adjusted to pH 7.5 with HCl, and containing 1 mM theophylline in order to block phosphodiesterase activity. Each syringe was rinsed with a further 5 ml of SM 7.5/300 which were added to the corresponding squashed follicles in 10 ml glass tubes. These were rapidly vortexed and centrifuged 10 min at 3600 $\times$ g. The following steps are

adapted from Meurs *et al.* (1980): to 2 ml of the intermediate phase (between the pellet and the floating lipids) were successively added 1 ml EDTA (0.04 M) and 1 ml HCl (0.8 M) in 5 ml glass tubes. After mixing, the tubes were placed in a boiling water bath for 3 min, cooled on ice, and centrifuged to eliminate the denaturated protein. An excess of CaCO_3 was added to neutralize the supernatant and the tubes were incubated for 1h at room temperature. CaCO_3 was then removed by centrifugation and the supernatant stored frozen (-20°C) until subsequent assay.

Determination of cyclic AMP

Binding protein was isolated from rabbit skeletal muscle and purified essentially as described by Gilman (1970) and modified by Tovey *et al.* (1974). The assay incubation and the separation of bound from free cAMP were performed in the same conditions as Meurs *et al.* (1980). Following observation by Tovey *et al.* (1974) that the assay is insensitive to large increases in salt and buffer concentrations, the assay buffer concentration was raised to 200 mM Tris-HCl, (pH 7.5), containing 4 mM EDTA, in order to equalize possible pH heterogeneity of CaCO_3 -neutralized samples.

Material

4-Pregnen- 17α , 20β -diol-3-one (17α , 20β -OH-P), 4-pregnen- 17α -ol-3,20-dione (17α -hydroxyprogesterone), 4-pregnen- 17α , 21-diol-3,20-dione (11-desoxycortisol), 4-pregnen-3,20-dione (progesterone), 4-pregnen- 20α -ol-3-one (20α -dihydroprogesterone) and 4-pregnen- 17α , 20α -diol-3-one (17α , 20α -OH-P), were purchased from Steraloids (USA). 5β -Pregnan- 3β , 17α , 20β -triol and 5β -pregnan- 17α , 20β -diol-3-one were a generous gift from Prof. Bun-Ichi Tamaoki (Japan). 4-Pregnen- 20β -ol-3-one (20β -dihydroprogesterone), 4-pregnen- 11β , 17α , 20β , 21-tetrol-3-one (20β -dihydrocortisol), 4-pregnen- 17α , 20β , 21-triol-3,11-dione (20β -dihydrocortisone) and 4-pregnen-3, 11, 20-trione were purchased from Sigma. 4-Pregnen-

21-ol-3,20 dione-(11-desoxycorticosterone) was from IBF Soc. Chim. (France).

Theophylline (1,3-dimethylxanthine), 3-isobutyl-1-methylxanthine (IBMX) and cholera toxin were obtained from Sigma, and forskolin from Calbiochem-Behring.

Adenosine 3': 5'-cyclic monophosphate (cAMP) and N⁶, 02'-dibutyryl-adenosine 3': 5'-cyclic monophosphate (db-cAMP) were purchased from Sigma.

Results

Relative efficiency of different steroids for induction of intrafollicular oocyte maturation in rainbow trout

The relative efficiency of different steroids compared with 17α , 20β -OH-P, as the ratio between the MED of 17α , 20β -OH-P (multiplied by 10^{-3}) and the MED of other steroids is summarized in Table 1. The data were obtained from several incubations, each one with follicles from one female, performed in the same experimental conditions. 17α , 20β -OH-P appears always to be the most potent, followed by other compounds identified in the ovaries of the ayu, *Plecoglossus altivelis*, as metabolites (Suzuki *et al.* 1981). The four naturally-occurring steroids at the top of the hierarchy exhibit the 17α , 20β diol function, which seems to have a physiological importance, since 17α , 20β -OH-P does not exhibit any activity.

The data shown in Table 2 suggest that the ranking of steroids according to their MED for intrafollicular oocyte maturation was different from one female to the next: 5β pregnan- 3β , 17α , 20β triol which exhibits a small MED, in the same order of magnitude as 17α , 20β -OH-P and smaller than 5β pregnan- 17α , 20β -diol-3 one in female 1, is much less efficient in female 2, with a MED much larger. However 17α , 20β -OH-P remained the most efficient steroid throughout.

Table 1. Relative efficiency of different steroids (progestins and corticosteroids) for induction of intrafollicular oocyte maturation *in vitro* in rainbow trout

* 4-pregnen-17 α , 20 β -diol-3-one (17 α , 20 β -OH-P)	1000
* 5 β -pregnan-3 β , 17 α , 20 β -triol	440 (2)
* 5 β -pregnan-17 α , 20 β -diol-3-one	270 (2)
4-pregnen-20 β -ol-3-one (20 β -dihydroprogesterone)	160 (1)
* 5 β -pregnan-3 α , 17 α , 20 β -triol	120 (1)
* 4-pregnen-17 α -ol-3,20-dione (17 α -hydroxyprogesterone)	40 (2)
4-pregnen-11 β , 17 α , 20 β , 21-tetrol-3-one (20 β -dihydrocortisol)	21 (1)
4-pregnen-17 α , 20 β , 21-triol-3,11-dione (20 β -dihydrocortisone)	21 (1)
4-pregnen-17 α , 21-diol-3,20-dione (11-desoxycortisol)	11 (1)
* 4-pregnen-3,20-dione (progesterone)	11 (1)
4-pregnen-20 α -ol-3-one (20 α -dihydroprogesterone)	8 (1)
4-pregnen-21-ol-3,20-dione (11-desoxycorticosterone)	4 (1)
4-pregnen-3,11,20-trione	0 (1)
4-pregnen-17 α ,20 α -diol-3-one- (17 α , 20 α -OH-P)	0 (1)

* metabolites identified from incubation of Ayu (*Plecoglossus altivelis*) ovaries with labelled 4-pregnenes as precursors, by Suzuki *et al.* 1981.

() number of assays (different females)

Table 2. Median efficient dose of various natural steroids for induction of intrafollicular oocyte maturation (GVBD) *in vitro* in 2 different female rainbow trout (ng/ml)

	Female 1	Female 2
4-pregnen-17 α ,20 β -diol-3-one (17 α , 20 β -OH-P)	54	111
5 β -pregnan-3 β ,s17 α ,20 β -triol	67	1450
5 β -pregnan-17 α ,20 β -diol-3-one	155	580

Inhibition of 17 α , 20 β -OH-P induced GVBD by substances expected to rise intraoocyte levels of cAMP (Fig. 1)

cAMP and db-cAMP, added to the incubation medium 2h prior to 17 α , 20 β -OH-P inhibited the maturation process, depending on the nucleotide

Fig. 1. The effect of various concentrations of substances expected to rise intraoocyte levels of cAMP, on the % of 17 α , 20 β -OH-P – induced GVBD. Each drawing links data obtained on oocytes from the same female (25 oocytes at each concentration for each female).

concentration and on the female (Figs. 1a, and b). The lower concentrations required for complete inhibition of GVBD varied between 1 and 8 mM of cAMP and between 2 and more than 8 mM db-cAMP.

Inhibitors of phosphodiesterase (i.e. IBMX and theophylline) added in the same way, also inhibited oocyte maturation (Figs. 1c and d). Inhibitory concentrations ranged between 0.012 and 0.11 mM of IBMX and 0.1 and 1 mM of theophylline, depending on the female.

Finally, substances known to stimulate adenylate cyclase, cholera toxin and forskolin, administered in the same way, had a strong inhibitory effect above doses ranging between 11 nM and more than 100 nM of cholera toxin, and between 1×10^{-3} mM and 33×10^{-3} mM of forskolin, depending on individual female (Figs. 1e and f).

In neither case was it possible to correlate oocyte

Fig. 2. Correlation between intraoocyte cAMP concentration and the mean follicular weight in females at various oocyte stage. For each female, intraoocyte cAMP and follicular weight were obtained from one sample of 10 follicles. The correlation coefficient $R = 0.59$ is highly significant at $p < 0.01$.

sensitivity of individual females to the inhibitory potency of these substances.

Intraoocyte cAMP concentration as a function of oocyte stage and sensitivity

Intrafollicular oocytes sampled by abdominal stripping, from end of vitellogenesis (EV) up to peripheral germinal vesicle stage (P-GV), exhibited a large variation of weight between individual females. Intraoocyte cAMP concentration, although also variable, appeared to be highly correlated to the mean follicular weight of individual females (correlation coefficient $R = 0.59$, significant at $p < 0.01$) (Fig. 2). Concerning morphological stages as defined by GV position, the mean cAMP concentrations at stages SP-GV (2.31 pmol/mg oocyte) or P-GV (2.05 pmol/mg oocyte) were significantly higher ($p < 0.01$) than at stages EV (0.84 pmol/mg oocyte) (Table 3). Finally, intraoocyte cAMP concentration appeared negatively correlated with the MED of $17\alpha, 20\beta$ -OH-P (correlation coefficient -0.57 , highly significant at $p < 0.01$) (Fig. 3).

Table 3. Comparison of cAMP concentration (pmol/mg oocyte) in rainbow trout oocytes at different morphological stages

Stage	Number of fish	Mean	Standard deviation
EV	44	.84	.75
SP-GV	23	2.31*	2.09
P-GV	12	2.05*	1.30

* significantly different ($p < 0.01$) from the value at stage EV; EV, end of vitellogenesis; SP-GV, subperipheral germinal vesicle; P-GV, peripheral germinal vesicle

Evolution of the cAMP content of oocytes incubated in vitro

a. Spontaneous fluctuations and effect of $17\alpha, 20\beta$ -OH-P (Fig. 4)

Six separate incubations were performed using intrafollicular oocytes from 6 different females, and groups of oocytes were sampled at increasing incubation times for each female. Two experiments were scheduled in order to look for the possible occurrence of cAMP fluctuations within 1h after the addition of $17\alpha, 20\beta$ -OH-P (3×10^{-6} M) (oocyte samplings after 0.5 sec, 1, 2, 3, 4, 6, 10, 15, 20, 30 and 40 min). Since no significant variations could be detected (data not shown), four other incubations were scheduled over a 10h period (samples taken every 45 or 60 min). The data presented in Fig. 4 were analysed by 2 factors (time/treatment) variance analysis. cAMP content exhibited large significant fluctuations during incubation time, but there was no consistent period or magnitude.

In all cases, the addition of $17\alpha, 20\beta$ -OH-P (3×10^{-6} M) to the incubation medium decreased intraoocyte cAMP within the first 10h of incubation. The situation and the duration of the diminution were differed amongst females. However, 2 factors variance analysis demonstrated that at least one part of the decrease was significant or highly significant in all females (Fig. 4).

Fig. 3. Correlation between intraoocyte cAMP concentration and the MED of 17α , 20β -OH-P for GVBD in different females. The correlation coefficient $R = -0.57$ is highly significant at $p < 0.01$.

Fig. 4. Evolution of cAMP as a function of incubation time in oocytes incubated *in vitro* within their follicle: —○— control; —●— 17α , 20β -OH-P, 3×10^{-6} M. a, b, c, d represent distinct data obtained with oocytes from 4 different females. Each point represents the mean cAMP content ($\pm$ SEM) of 3 samples of 10 oocytes (female a) or of 5 samples of 10 oocytes (females b, c, d). Significant differences between control and 17α , 20β -OH-P treatment are indicated above the corresponding incubation time (differences significant at the probability level $p = 0.05$ (☆), $p = 0.01$ (♂), $p = 0.001$ (♂)).

b. Effect of IBMX and IBMX + forskolin on the accumulation of intraoocyte cAMP (Fig. 5)

Three sets of experiments were carried out with follicles from 3 females. Only data concerning 2 of

Fig. 5. Accumulation of cAMP (expressed in % of initial control) in intrafollicular oocytes incubated *in vitro* in the presence of IBMX (a) or IBMX + forskolin (b). Each drawing links data obtained in oocytes from distinct females. Percentage are calculated from cAMP contents obtained in triplicate samples of 5 oocytes.

them are represented in Fig. 5a, the other being intermediate. In two cases a small significant but transient increase of cAMP was observed after 1h incubation with IBMX (1 mM), followed by successive fluctuations in the same order of magnitude. In one case no increase in cAMP content could be detected before 35 to 48h of incubation.

When forskolin was added to the medium in addition to IBMX (Fig. 5b), a large accumulation of cAMP occurred in the oocytes, within a few hours. In one female, forskolin (0.05 mM) in addition to IBMX (1 mM) induced a rapid increase of cAMP (15 times the initial content after 1h) with a maximum after 5h (30 times the initial content) followed by a rapid decrease. In another female, a lower concentration of forskolin (0.01 mM) was still able to multiply by 6 fold the initial level after 6h incubation. In both cases a rapid decrease occurred soon after the maximum level was observed but did not reach the initial level even after 20h.

Discussion

The results presented here concerning the efficiency of different steroids for induction of oocyte maturation, including 5β -reduced metabolites recently identified in incubations with labelled 17α , 20β -OH-P of ovaries of the ayu *Plecoglossus altivelis* (Suzuki *et al.* 1981), show that 17α , 20β -OH-P always appears to be the most efficient steroid for induction of intrafollicular oocyte maturation in trout. In contrast, the ranking of other steroids according to their relative efficiency in comparison with 17α , 20β -OH-P can vary from one female to the other. This probably reflects differences either in the functional maturity of the follicular layers for metabolization of closely related steroid molecules, or in the functional maturity of the oocyte itself to respond to a signal more or less specific. Thus, taking into account the fact that 17α , 20β -OH-P is the most efficient naturally-occurring steroid for induction of intrafollicular oocyte maturation *in vitro*, that it is able to trigger GVBD even in defolliculated oocytes (Jalabert 1976), and that it can be found at high concentrations in the plasma around the time of natural maturation and ovulation (Campbell *et al.* 1980, Fostier *et al.* 1981a) or secreted *in vitro* by follicles stimulated by GtH (Fostier *et al.* 1981b), it may be assumed that 17α , 20β -OH-P is certainly the main specific MIS in the rainbow trout and can probably be considered as the principal MIS at least in the suborder salmonoids (Nagahama *et al.* 1983; Nagahama and Adachi 1985).

Concerning the intraoocyte mechanisms following the action of 17α , 20β -OH-P, our data show that all the substances expected to induce an increase in the intracellular level of cAMP (cAMP and dbcAMP, phosphodiesterase inhibitors theophylline and IBMX, adenylate cyclase stimulators cholera toxin and forskolin) effectively block 17α , 20β -OH-P-induced GVBD *in vitro*. A similar effect was obtained in *Salvelinus fontinalis* intrafollicular or denuded oocytes, and in intrafollicular oocytes of yellow perch (Goetz and Hennessy 1984; De Manno and Goetz, unpublished data), using forskolin and the phosphodiesterase inhibitors IBMX and SQ 20, 006. According to Wells and Kramer

(1981) the specificity of phosphodiesterase inhibitors could be questioned. In fact our own results show that cAMP accumulation after IBMX is not constant, depending on the female, and is of weak magnitude, even though GVBD is completely inhibited. Such a weak effect of phosphodiesterase inhibitors upon cAMP accumulation was also found in amphibian oocytes (O'Connors and Smith 1976; Bravo *et al.* 1978, Mulner 1983). In trout, as well as in amphibians, this might be due either to a lack of specificity of methylxanthines which could block GVBD by interfering in other steps of the maturation process, or to other possibilities, such as a slow turn over rate of free cAMP in the oocyte, most part of cAMP being bound to proteins, or because some kinds of phosphodiesterases are unsensitive to methylxanthines (Weiss *et al.* 1979). The inhibitory effect of forskolin upon trout oocyte maturation appears much more demonstrative of an action *via* adenylate cyclase stimulation, considering the large cAMP accumulation observed in the oocytes after only 1 to 5h of incubation. In our conditions this accumulations is much more important than in *Xenopus* (Schorderet-Slatkine and Beaulieu 1982). After reaching a peak within a few hours, intra-oocyte cAMP level decreases rapidly in trout without reaching again the initial concentration. This decrease might be due to the fact that IBMX was unable to block completely phosphodiesterases activity, or to a leakage of cAMP from the oocyte into the incubation medium, or both, associated probably with a depletion of ATP as available precursor for cAMP. Finally, cAMP and db-cAMP directly added into the incubation medium block 17α , 20β -OH-P induced GVBD in trout. A surprising point however, is that cAMP appears more efficient in inhibiting intrafollicular oocyte maturation in trout than its dibutyryl derivative. This observation contradicts data obtained in amphibians (Morrill *et al.* 1977) and in mammals (Magnusson and Hillenjo 1977). On the other hand, we know that db-cAMP is more efficient than cAMP in the promotion of 17α , 20β -OH-secretion by trout follicles *in vitro* (Fostier, A., unpublished data). This could mean that the oocyte cell is more permeable to cAMP than the follicular

cells, which might be of physiological importance to understand the regulation of oocyte sensitivity to the MIS within the follicular complex.

We also demonstrate here that the induction of oocyte maturation *in vitro* is accompanied by a decrease in oocyte cAMP within the first 10h of incubation. This decrease is weak, with an irregular kinetics, but was found in all the four experiments performed. In fact, this phenomenon was controversial for some time in amphibians, probably because of the small magnitude of the decrease compared to the whole amount of oocyte cAMP, and limitations in the precision of available assays, as underlined by Ciccirelli and Smith (1985). The reason why we were able to observe the cAMP decrease after 17α , 20β -OH-P action in trout oocytes, sometimes at the limit of significance, is probably because of the large number of oocytes used for each sample (10 or 5) and the use of repeated samples (usually 5) for each time of incubation.

Considering the results discussed above, showing that an artificially-induced cAMP increase inhibits trout oocyte maturation, and that the action of 17α , 20β -OH-P is accompanied by some transitory decrease in cAMP level, other of our observations might appear contradictory. Thus, we observed that follicular growth (essentially due to oocyte growth) is highly correlated with an increase in oocyte cAMP concentration. Also GV migration, which occurs at the end of oocyte growth and is known to be associated with an increase in oocyte sensitivity to 17α , 20β -OH-P (Jalabert and Fostier 1984a), is associated with a significant rise in oocyte cAMP concentration. Moreover, there is a negative significant correlation between oocyte cAMP concentration and the MED of 17α , 20β -OH-P for oocyte maturation, which means that oocyte sensitivity is positively correlated with cAMP concentration. This is a very different situation to that in *Xenopus* where such correlation was not observed (Mulner 1983). In trout, as in *Xenopus*, the oocyte cAMP level, most of which is probably bound to proteins such as the regulatory subunit of cAMP dependent protein kinase, is the result of a dynamic equilibrium between synthesis by adenylate cyclase and degradation by phosphodiesterase. It is pos-

sible, however, that the enzymatic activities which control the cAMP level and its rate of turn over, may be very different in laboratory-reared *Xenopus* which are kept for long periods with full-grown ovaries in such conditions that oocyte maturation and ovulation are delayed, and in rainbow trout which exhibits a clear cut seasonality and in which maturation and ovulation occur annually in continuity with the end of oocyte growth. Therefore, the rainbow trout might offer an original model with which to study how oocyte sensitivity to hormones is progressively set up in lower vertebrates exhibiting a clear cut seasonal ovulation.

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