Sulfation and uptake of the maturation-inducing steroid, 17α,20β-dihydroxy-4-pregnen-3-one by rainbow trout ovarian follicles

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Accepted: February 4, 1995

Abstract

Rainbow trout ovarian follicles were incubated in vitro with tritiated 17α,20β-dihydroxy-4-pregnen-3-one (17,20β-P; maturation-inducing steroid). Within 18–24 h, 56–66% had been converted to tritiated 17α,20β-dihydroxy-4-pregnen-3-one 20-sulfate (identification confirmed by HPLC) and 27% had been taken up (absorbed) by the follicles. Addition of 125 ng of cold (non-tritiated) 17,20β-P to the incubations caused a decrease in the percentage of [³H]-17,20β-P which was sulfated (56% – 10%) and an increase in the percentage that was taken up (27% – 57%). Seven steroids were tested for their effectiveness in decreasing the sulfation and increasing the uptake of tritiated [³H]-17,20β-P. The order of effectiveness was in both cases the same: 17,20β-P > cortisol > 11-deoxycortisol > 17α,20β,21-trihydroxy-4-pregnen-3-one > 17α-hydroxy-4-pregnen-3,20-dione > 17β-estradiol > testosterone. This indicated that the processes of sulfation and uptake of [³H]-17,20β-P were related to each other and led to the hypothesis that, when cold 17,20β-P is added to the medium, it reduces the proportion of [³H]-17,20β-P which is sulfated and thus allows more free [³H]-17,20β-P to enter the ovarian follicles. This hypothesis was supported by the finding that each ovarian follicle had the capacity in vitro to sulfate only ca. 2 ng of [³H]-17,20β-P per 18h but a capacity to take up > 500 ng per 18h.

Gonadotropin I, Gonadotropin II, forskolin and phorbol-12-myristate-13-acetate (which all have an affect on steroid biosynthesis) did not affect the amount of 17,20β-P which was sulfated. Sulfating activity was localized in the thecal cell layer of the follicle. The yolk fraction was shown to be responsible for absorbing the [³H]-17,20β-P.

Introduction

It has been well-established that the process of oocyte maturation in rainbow trout is induced by the steroid 17α,20β-dihydroxy-4-pregnen-3-one (17,20β-P), which is produced within the ovary in response to gonadotropin II (GtH II) stimulation (see review by Nagahama 1987). It has also been well-established that the main site of production of 17,20β-P is within the follicular cell layers which surround the oocytes; the outer thecal cell layer producing 17α-hydroxy-4-pregnen-3,20-dione (17-P) and the inner granulosa cell layer converting this 17-P to 17,20β-P (Young et al. 1986).

Despite the large number of studies which have been carried out on the in vitro and in vivo production of 17,20β-P in rainbow trout and other salmonids since 1981 (when the first radioimmunoassays for this steroid became available), it was not until 1991 that it was discovered that rainbow trout
ovaries, in addition to synthesizing unmodified free steroid, also synthesized substantial amounts of sulfated 17,20β-P (Scott and Turner 1991). It has subsequently been established that this latter compound, 17α,20β-dihydroxy-4-pregnen-3-one 20-sulfate (17,20β-P-sulfate) is hydrophilic, strongly anionic and resistant to hydrolysis by “sulfatase”-containing enzyme preparations, but can be converted to free 17,20β-P by acid solvolysis (Scott and Canario 1992). It is excreted by females in large amounts (via the urine) both during and after oocyte maturation and ovulation (Scott and Liley 1994), and is also produced by testes of spermiating males (Scott and Turner 1991). Concerning its role, it does not have any oocyte maturation-inducing activity but has been shown to be a potent odorant in another salmonid, the Atlantic salmon, Salmo salar (Moore and Scott 1992) and in goldfish, Carassius auratus (Sorensen et al. 1991); which suggests that it might have a role as a pheromone.

Based on mammalian studies, it is almost certain that the sulfation of 17,20β-P in the rainbow trout ovary is brought about by an enzyme known as a sulfotransferase (see review by Scott and Vermeersen 1994). The present study was undertaken to establish the speed, capacity and specificity of action of this enzyme (which we have tentatively termed “20β-hydroxysteroid sulfotransferase”) in intact rainbow trout ovarian follicles and also to determine in which of the follicular cell layers the enzyme might be localized.

During the course of the study, it was discovered that intact ovarian follicles had a tendency to take up (absorb) between 27 and 57% of the radioactive precursor ([3H]-17,20β-P) which had been added to the medium. Since this appeared to have a direct influence on the percentage of [3H]-17,20β-P which could be sulfated, this phenomenon was also investigated.

Materials and methods

The majority of experiments were carried out in Okazaki, Japan, using three-year old fish obtained directly from a trout hatchery. The experiments which were designed to examine the effect of different steroids on the degree of sulfation and uptake of 17,20β-P were carried out at Lowestoft, using two-year old fish which had been obtained from a local trout farm 3 weeks prior to the experiments. Most experiments used intact ovarian follicles prepared and incubated as described by Kagawa et al. (1982). In some experiments, thecal and granulosa cell layers were manually separated using fine forceps according to the methods described by Kagawa et al. (1982). In other experiments, yolk was gently extruded from the follicles (“yolk-extruded follicles”) prior to incubation. All follicles were at a stage where the germinal vesicle had migrated close to the periphery of the oocyte but had not yet started to break down. The incubations were in polystyrene flat-bottomed, 12 well, tissue culture plates. The incubation medium, trout balanced salt solution (TBSS), consisted of: NaCl, 8.60 g; KCl, 0.23 g; MgSO₄·H₂O, 0.07 g; MgCl₂, 0.20 g; CaCl₂, 0.50 g; HEPES, 0.95 g; glucose, 1 g; distilled water, 1 l; adjusted to pH 8.0 with 1M NaOH. The majority of experiments were carried out in Japan using three-year old fish and, in all cases, 5 follicles (or 5 isolated cell layers) were placed in each well with 2 ml of TBSS. The specificity experiments were carried out in the UK using two-year old fish and because these fish had smaller eggs (ca. 4 mm as opposed to 5 mm diameter), 8 follicles were placed in each well with 2 ml of TBSS. All treatments were carried out in triplicate. Each well contained ca. 50,000 dpm (70 pg) of [3H]-17,20β-P, which was added in 10 μl of ethanol. Three wells containing [3H]-17,20β-P and incubation medium only were included in each experiment. Depending on the experiment, non-radioactive steroids (concentrations varying from 8 to 250 ng) and forskolin (to yield a final concentration of 10 μM) were added to the wells in 10 μl ethanol; chum salmon GtH I (2 μg) and GtH II (2 μg) were added in 20 μl TBSS; phorbol-12-myristate-13-acetate (PMA; to yield a final concentration of 100 nM) was added in 5 μl of dimethyl sulfoxide (DMSO); appropriate volumes of ethanol, TBSS and DMSO were added to the control groups of follicles. Follicles were routinely incubated for 18h at 10°C.

At the end of the incubation period, the medium