EVIDENCE FOR A POSSIBLE MEDIATORY ROLE OF TUMOR NECROSIS FACTOR α ON LUTEINIZING HORMONE-INDUCED OOCYTE MATURATION IN TROUT

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Introduction:
In fish, like in other vertebrates, luteinizing hormone (LH) is an essential hormone for the completion of oocyte maturation. In salmonid fish (i.e., salmon and trout), oocyte maturation is induced by LH through its stimulation of the production of the maturation-inducing steroid, 17α,20β-dihydroxy-4-pregnen-3-one (17,20β-P). Specifically, LH stimulates the production of 17,20β-P through the production of its steroidal precursor 17α-hydroxyprogesterone (17-OHP) as well as the expression and activity of 20β-hydroxysteroid dehydrogenase (20β-HSD), the enzyme responsible for the conversion of 17-OHP to 17,20β-P. In mammals, several factors have been reported to modulate the effects of LH on oocyte maturation. For example, a strong body of evidence suggests that tumor necrosis factor α (TNFα) could play multiple physiological roles in the control of ovarian function. In the present study, we have investigated the possible involvement of TNFα in the regulation of oocyte maturation in the brown trout (Salmo trutta).

Methods:
After dissection, brown trout ovaries were placed in Hank’s balanced salt solution (HBSS) and individual ovarian follicles were manually separated with forceps from each ovary on ice, as previously described [1]. For the in vitro induction of GVBD, brown trout follicles at the preGVBD stage were incubated in HBSS containing 0.2% BSA (HBSS-BSA). Ten follicles were placed in each well of a 6-well culture plate containing 4 ml of HBSS-BSA in the absence or presence of the test compounds for 48 h at 15°C with shaking. At the termination of the incubation period the culture medium was removed and stored at -20°C until assayed. Ovarian follicles after treatment with the test compounds, as previously described [2]. Subsequently, ovarian tissues were flash frozen in liquid nitrogen and stored at -80°C until assayed.

Results and Discussion:
Our results show that in vitro treatment of brown trout preovulatory follicles with coho salmon LH (sLH) significantly increased oocyte maturation, as assessed by germinal vesicle breakdown (GVBD), and this effect was blocked by TAPI-1 (an inhibitor of TNFα converting enzyme or TACE/ADAM17). Furthermore, treatment of trout preovulatory follicles with sLH increased the expression of Tnfα. Interestingly, recombinant trout TNFα (rtTNFα) significantly increased the number of follicles undergoing GVBD. Our results also show that the stimulatory effects of rtTNFα on oocyte maturation were the result of the direct involvement of rtTNFα in stimulating the production of 17,20β-P as evidenced by the complete blockage of sLH-stimulated production of both 17-OHP and 17,20β-P by TAPI-1 and by the direct effects of rtTNFα in stimulating the production of 17,20β-P and the expression of 20β-hsd. Interestingly, sLH and rtTNFα also increased the ovarian expression of the LH receptor (Lh-r).

Conclusion:
These results strongly suggest that TNFα may contribute to the regulation of oocyte maturation by LH in trout.

References: