HORMONAL REGULATION OF *AQUAPORIN-1ab* IN *HETEROPNEUSTES FOSSILIS* OOCYTES *IN VITRO*

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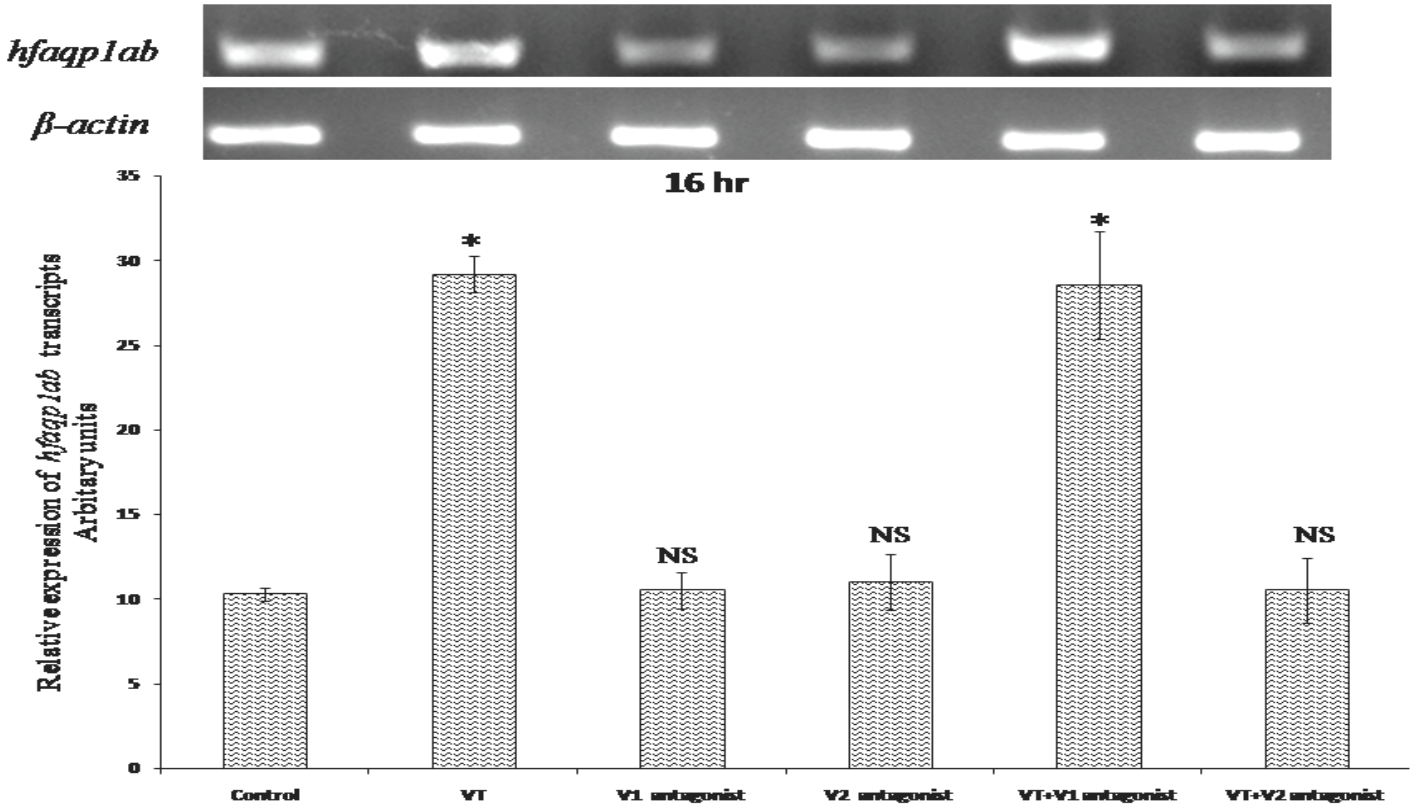
Introduction:

In teleosts, the maturing oocytes undergo swelling and a significant increase in volume due to water uptake prior to ovulation. This physiological process called oocyte hydration was first described in marine teleosts. Aquaporins (AQPs) are selective water or water and solute transporting membrane channels that have been remarkably conserved throughout evolutionary history [1]. In mammals, 13 AQP isoforms (AQP0-AQP12) have been identified [4]. The functions and physiological actions of AQPs have received extensive attention in humans and some other mammals. However, in non mammalian vertebrates such as fish, much less is known about AQP distribution, function and regulation [2]. The oocytes of the freshwater catfish *Heteropneustes fossilis* hydrate during hormone-induced meiotic maturation [5]. Recently, we cloned and characterized an ortholog of the teleost aquaporin-1ab in catfish (HfAqp1ab), whose transcripts were detected exclusively in ovary and brain, and followed a seasonal expression pattern [3]. In ovarian follicles, *hfaqp1ab* expression was stimulated during vasotocin (VT) and hCG-induced final oocyte maturation and hydration. In the present investigation, the response of VT was compared to that of 17α, 20β-hydroxy-4-pregnen-3-one (17,20βP, a maturation-inducing steroid in the catfish), and the type of VT receptor involved was investigated.

Methods:

Acclimatized catfish were sacrificed in the late prespawning phase by decapitation and ovaries were removed, weighed, and transferred into sterile culture plates containing incubation medium alone or with optimized doses of 17,20βP (1µg/ml), VT (Arg-8-oxytocin) acetate salt (100nM), and arginine-vasopressin



Expression of *hfaqp1ab* in oocyte after16 hours of incubation. The relative densitometric values of the PCR product bands from four gels are presented as mean ± SEM and analyzed by one way ANOVA followed by Tukeys' test (P<0.05)

V1 receptor antagonist deamino Pen[1], O-Me-Try[2], Arg[8] (10^{-6}M) and V2 receptor antagonist 1-adamantaneacetyl-O-Et-D-Try[2],Val[4],Abu[6], Arg[8,9] (10^{-6}M). The oocyte incubation protocol was that described by Singh and Joy [5]. Oocytes were sampled at 8, 16 and 24 h and processed for *hfaqp1ab* gene expression by semi-quantitative RT-PCR.

Results and Discussion:

The expression of *hfaqp1ab* increased when the ovarian follicles were incubated with VT or 17,20βP in relation to the control groups. The expression was higher after 16 h of incubation with VT and resulted in higher transcript levels than with 17,20βP. At 24 h, the expression decreased to the control levels. However, when the follicles were incubated with both VT and 17,20βP no synergistic effect was found, rather the expression was low and inverse with the time period, suggesting an early saturation of the transcriptional process by the combined stimulus. The V1 type receptor antagonist did not inhibit the VT-induced *hfaqp1ab* gene expression, rather this was enhanced at 8 h or was unchanged at 16 and 24 h. (See Fig) In contrast, co-incubation of VT with the V2 receptor antagonist inhibited the induction of *hfaqp1ab* expression by VT, suggesting that the V2 type receptor may be involved in the mechanism of VT-mediated oocyte hydration. Thus, the V2 receptor may control the expression of HfAqp1ab during oocyte hydration, similarly as it occurs for the vasopressin-dependent mammalian AQP2 which is regulated via the V2 type receptor. This finding strengthens the hypothesis that although *hfaqp1ab* belongs structurally to the AQP1 subfamily of water channels, it shows functional similarities with amphibian AQP-h2 and Japanese quail and mammalian AQP2 [3]. The V1 type receptor, which activates the IP3-PKC pathway, may not be directly involved in oocyte hydration, or the stimulation of *hfaqp1ab* gene expression may be consequential to the suppression of the IP3-PKC pathway triggering some yet unknown mechanism.

Conclusion:

These present results indicate that VT stimulates *hfaqp1ab* gene expression in catfish ovarian follicles through the V2 receptor type, being more effective than 17,20βP.

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