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An *in vitro* study on noradrenergic modulation of final oocyte maturation in the catfish *Heteropneustes fossilis*

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ABSTRACT

This study was aimed to elucidate *in vitro* the effects of endogenous catecholamines: dopamine (DA), noradrenaline (NA) and adrenaline (A), and the β-adrenergic blocker propranolol on induction of final oocyte maturation (FOM) in the catfish *Heteropneustes fossilis*. With this aim, post vitellogenic follicles from sexually mature gravid female catfish were incubated with each of DA, NA and A in a concentration range of 5–250 μM, and propranolol in a concentration range of 1–200 μg/mL at time points varying from 0 to 30 h). Translucent follicles without germinal vesicle (GV) and opaque follicles with GV were scored separately for the calculation of percentage germinal vesicle breakdown (GVBD), an index of FOM. Data were analyzed by one-way ANOVA and were considered statistically significant when P values were less than 0.05. The analysis of the data showed that the incubation with NA only stimulated GVBD in a concentration - and time-dependent manner. Though the incubation with propranolol decreased total follicular cAMP level significantly at and above 10 μg/mL concentrations, a significant effect of the GVBD increase was noticed at 50 μg/mL or higher. However, the 10 μg/mL concentration of propranolol was effective to inhibit the NA-induced GVBD significantly albeit at a low level (39%). The present study suggests that final oocyte maturation is modulated by NA through a β-adrenergic mechanism, implicating a neural control of oocyte maturation and ovulation in teleosts.

1. Introduction

In vertebrates, oogenesis and ovulation are primarily regulated by the sequential interplay of endocrine hormones of the brain–pituitary-ovary axis. Ovarian innervation of sympathetic and sensory nerves of the peripheral nervous system has been documented for mammals [1–5], but not for lower vertebrates. Evidence of a neural involvement, alone or in synergy with gonadotropins and other hormones, in different follicular functions has been reported in mammals [5]. The presence of catecholamine neurons has been demonstrated in the ovary and oocytes of primates [4,7]. In these studies, noradrenaline (NA) has been reported as the major ovarian catecholamine neurotransmitter. NA is implicated in ovarian development, steroidogenesis and folliculogenesis, and in ovulation and corpus luteum function [5–10].

Recent studies from our laboratory have presented evidence for catecholaminergic innervation of the catfish ovary [11–13]. The ovarian nerves are traced to the spino-sympathetic system. At least some of the ovarian nerves are tyrosine hydroxylase (TH) - immunoreactive, and seasonal and periovulatory changes in ovarian TH activity and catecholamines have been reported. Thus a functional role of the catecholaminergic system in ovarian activity may be implied. We also reported that catecholamines may elicit differential effects: DA activity (turnover) was low at 8 h post hCG injection (coinciding with oocyte maturational response) but NA activity (turnover) was maintained high up to 16 h of hCG injection, coinciding with the release of eggs after stripping [11,12]. Chaube and Joy [14] reported that the TH inhibitor (α-methyl-paratyrosine, α-MPT) inhibited the hCG-induced spawning response in the catfish. Furthermore, catecholamines exert differential and stage-specific effects on ovarian steroidogenesis which may suggest a functional interaction between catecholamines and gonadotropin [15]. All the above observations indicate a greater involvement of catecholamines in fish ovarian functions.

Induced breeding is an integral part of fish culture, the world over and hypophysation (application of pituitary extract or purified

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gonadotropins such as LH and hCG) has been practiced since 1930's. The use of exogenous gonadotropins and gonadotropin-releasing hormone (GnRH) analogue, alone or in combination with dopamine (DA) receptor (DA_2) antagonist, are recent advancements in this direction [16–19]. LH stimulates final oocyte maturation (FOM) by inducing a steroidogenic shift in the post vitellogenic follicles causing a simultaneous inhibition of estradiol-17β (E_2) and stimulation of a maturation-inducing hormone (MIH), which is identified to be 17,20β-dihydroxy-4-pregnen-3-one (17,20β-DP) in the catfish [20]. The changes in the hormonal milieu leads to inhibition of follicular/oocyte cAMP that maintains the meiotic arrest [17–23]. While the downstream mechanisms underlying meiotic maturation is well documented at the physiological and molecular level, the regulatory mechanisms controlling ovulation are poorly understood [16]. Haider and Baqri reported that β-adrenergic receptor blockers propranolol (1.5 mM) and alprenolol (1.0 mM) stimulated germinal vesicle breakdown (GVBD) in a time-dependent manner, coinciding with a significant decrease in oocyte cAMP levels [24]. These observations imply a functional role of naturally occurring β-adrenergic receptor agonists like NA and adrenaline in FOM. In this regard, the observation of Jalabert on the involvement of the sympathetic nervous system in the ovulation of rainbow trout oocytes *in vitro* is significant [25].

In the present study, we examined the effects of naturally occurring catecholamines and β-adrenergic receptor blocker propranolol on *in vitro* oocyte maturation in *H. fossilis*. The effect of propranolol on cAMP levels and GVBD response was compared. We also examined whether the stimulatory effect of NA could be blocked by the β-receptor blocker. The results suggest a potential role of NA on oocyte maturation and the involvement of a β-adrenergic receptor mechanism.

2. Materials and methods

2.1. Chemicals

Dopamine (DA), noradrenaline (NA), adrenaline (A) and propranolol (P) were purchased from Sigma (St. Louis, MO, USA). Cyclic AMP EIA kit was purchased from Cayman Chemical Co. USA. All other chemicals were of analytical grade and purchased locally.

2.2. Experimental location and facilities

The experiments were carried out in the aquarium facility of the animal house of the Department of Zoology, Banaras Hindu University, Varanasi. The experiments were performed in accordance with the guidelines of Banaras Hindu University for experimentation in animals and all care was taken to prevent cruelty of any kind.

2.3. Experimental animals and husbandry

Gravid female *Heteropneustes fossilis* (60–70 g) were purchased from a local fish supplier in June (pre-spawning phase) and stocked in the aquarium facility of the animal house. They were maintained in cement tanks under normal photoperiod (13.0L: 11.0D) and temperature (25 ± 2 °C) until used for experiments. Water was changed daily after feeding. The fish were fed goat liver daily *ad libitum*. A few female fish were sampled randomly to determine the maturity of the gonads. Fish and gonad weights were taken to calculate the gonado-somatic index (GSI), which was 9.58 ± 0.67. The ovaries were dissected and contained mature dark green postvitellogenic follicles (oocyte diameter 1.0 mm), which were used for the experiments.

2.4. Experimental design and preparation of incubation medium

The incubation medium used was the one described by Goswami and Sundararaj [26] and Upadhayaya and Haider [27], but with minor modifications. The incubation medium consisted of (in grams) NaCl 3.74, KCl 0.32, $CaCl_2$ 0.16, $NaH_2PO_4 \cdot 2H_2O$ 0.10, $MgSO_4 \cdot 7H_2O$ 0.16 and glucose 0.40 in 1 L of triple distilled water. As phenol red (indicator of pH) is estrogenic, it was omitted in the preparation of the medium. The pH was adjusted to 7.5 with 1 N sodium bicarbonate and autoclaved. Penicillin (200,000 Units) and streptomycin sulphate (200 mg) were added and filtered. The medium was stored at 4 °C and was prepared fresh every week. The medium was stored in a refrigerator.

For oocyte collection, catfish was sacrificed by decapitation and ovaries were transferred to a sterile petri dish containing fresh cooled incubation medium. Dark green postvitellogenic follicles were separated from each other with the help of fine brush and watchmaker forceps. Batches of 30–40 follicles were incubated at 25 ± 2 °C in embryo cups containing each of 3 mL plain medium (control), medium containing various test compounds (treated) or medium containing vehicle (vehicle control) for different intervals (duration study) or for 24 h. The experiments were replicated with follicles collected from five fish. At the end of the incubations, the follicles were cleared in a clearing solution (ethanol: acetic acid: formalin; 6:1:3) and examined under a stereo binocular microscope for the GVBD assay. Translucent eggs without GV and those containing GV were counted separately and percentage was calculated from the total number of the follicles incubated. Follicular cAMP level was assayed by an ELISA kit.

2.5. Experimental treatments

2.5.1. Effects of catecholamines on oocyte maturation

To study the effects of different concentrations of catecholamines, stock solutions of DA, NA and A were prepared in 1 mM HCl (10–50 μL). Working concentrations (5, 10, 20, 50, 100, 200 and 250 μM) of each were prepared by diluting the stock solutions with the incubation medium, just before the incubations. The pH of the incubation medium was adjusted to 7.4. About 30–40 follicles were incubated in triplicate in each concentration of DA, NA or A. As control, the follicles were incubated in plain medium (negative control) or the medium containing the same volume of vehicle (positive control). After 24 h, the follicles were cleared for GVBD scoring, as described above. The incubations were replicated with samples from five fish.

To study the duration effect of NA, about 30–40 follicles in triplicate were incubated with 250 μM of NA for 0, 3, 6, 12, 24 or 30 h. The duration studies were not conducted for DA and A since they were ineffective in the concentration studies. As controls, the follicles were incubated similarly in plain medium with or without the vehicle. At the end of each interval, the follicles were transferred to the plain medium for incubation up to 30 h (except the 30 h group). At the end of the incubations, the follicles were cleared and GVBD was evaluated. The incubations were replicated with samples from five fish.

2.5.2. Effect of propranolol on oocyte maturation

A stock solution of propranolol (a β-adrenoceptor blocker) was prepared in 50 μL ethanol and diluted with the incubation medium to make different concentrations. About 30–40 follicles in triplicate were incubated in different concentrations (1, 10, 20, 50, 100 and 200 μg/mL) of the inhibitor. As controls, the follicles were incubated in plain medium (negative control) or in the medium containing the same volume of the vehicle (positive control). GVBD was evaluated after 24 h incubation. The incubations were replicated