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Role of catecholestrogens on ovarian prostaglandin secretion *in vitro* in the catfish *Heteropneustes fossilis* and possible mechanism of regulation

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ABSTRACT

Seasonal, periovulatory and 2-hydroxyestradiol-17β (2-OHE_2)-induced changes on ovarian prostaglandin (PG) E_2 and $F_{2\alpha}$ were investigated under *in vivo* or *in vitro* in the female catfish *Heteropneustes fossilis*. Both PGE_2 and $PGF_{2\alpha}$ increased significantly during ovarian recrudescence with the peak levels in spawning phase. The PGs showed periovulatory changes with the peak levels at 16 h after the hCG treatment. Incubation of postvitellogenic ovary fragments with estradiol-17β (E_2), 2-OHE_2 or 2-methoxyE_2 produced concentration-dependent increases in PG levels; 2-OHE_2 was more effective. In order to identify the receptor mechanism involved in the 2-OHE_2-induced PG stimulation, the ovarian pieces were incubated with phentolamine (an α-adrenergic antagonist), propranolol (a β-adrenergic antagonist) or tamoxifen (an estrogen receptor blocker) alone or in combination with 2-OHE_2. The incubation of the tissues with the receptor blockers alone did not produce any significant effect on basal PG levels. However, co- and pre-incubation of the tissues with the blockers resulted in inhibition of the stimulatory effect of 2-OHE_2 on the PGs. Phentolamine was more effective than propranolol. The signal transduction pathway(s) involved in the 2-OHE_2-induced PG secretion was investigated. The incubation of the ovarian pieces with 3-isobutyl-1-methylxanthine (IBMX, a phosphodiesterase inhibitor), chelerythrine (a protein kinase C inhibitor) and PD098059 (a mitogen-activated protein kinase inhibitor) significantly lowered the basal secretion of $PGF_{2\alpha}$ and PGE_2. In contrast, H89 (a protein kinase A inhibitor) increased the basal secretion of PGs at 1 and 5 μM concentration and decreased it at 10 μM concentration. The co- or pre-incubation with IBMX, H89, chelerythrine and PD098059 significantly inhibited the stimulatory effect of 2-OHE_2 on $PGF_{2\alpha}$ and PGE_2 levels. The inhibition was higher in the pre-incubation groups. Chelerythrine was the most effective followed by PD098059, IBMX and H89. The results suggest that 2-OHE_2 may employ both adrenergic and estrogen receptors, or a novel receptor mechanism having properties of both adrenergic and estrogen receptors.

1. Introduction

Involvement of prostaglandins (PGs) as local mediators of ovulation, fertilization, implantation and parturition is well established in mammals [3,4,28,32,40,46,61]. Among the PGs, both PGE_2 and $PGF_{2\alpha}$ are implicated in the regulation of reproductive processes in vertebrates and the latter appears to play a more critical role in ovulation [42,58]. In teleosts, PGs have been implicated with final oocyte maturation (FOM), steroidogenesis, sexual behavior and blood flow [15,19,23,29,44,50,55]. However, Jalabert and Szollosi [23] in rainbow trout (*Salmo gairdneri*) and Goetz et al. [16] in brook trout (*Salvelinus fontinalis*) reported differential effects of PGs; oocytes ovulated when exposed *in vitro* to $PGF_{2\alpha}$ but did not ovulate in the presence of PGE. Further, Cetta and Goetz [6] reported that ovarian PGE level declined prior to, and during ovulation but $PGF_{2\alpha}$ level remained high by the completion of ovulation or even after ovulation up to 24 h.

Both gonadotropins and steroid hormones are involved in the regulation of arachidonic acid (AA) metabolism and PG production. In mammals, gonadotropins stimulate follicular production of PGs [39] and the gonadotropin-induced surge in PG levels is regulated by progestins [4]. Under the gonadotropin surge, the expression of phospholipase A_2 (PLA_2) and PG synthesis enzymes in the granulosa cells is stimulated [11,12]. Similarly in teleosts gonadotropin, progestins and other steroids have been reported to modulate PG synthesis [18,21,41] although apparent species and ovary stage-specific variations were also described in the studies. In sexually mature zebrafish (*Danio rerio*), the ovulatory gonadotropin (hCG) surge activated the follicular AA pathway [30]. Further, it has been shown that activation of the protein kinase C (PKC) pathway and

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elevation of Ca^{++} level are stimulatory, while stimulation of the cyclic adenosine monophosphate (cAMP) pathway is inhibitory to PG production [24].

Catecholestrogens are estrogen metabolites, which have been implicated in reproductive functions such as embryo implantation, prostaglandin synthesis, gonadotropin release, parturition, increase in uterine weight, egg transport in cycling rats, ovarian follicular steroidogenesis and angiogenesis [1,43,52,54]. Recently seasonal and periovulatory changes in catecholestrogens and estrogen hydroxylase activity were demonstrated in the ovary of the catfish *Heteropneustes fossilis* [8,33]. Hydroxyestrogens have been shown to stimulate FOM and ovulation by stimulating the secretion of a maturation-inducing steroid (17, 20β-dihydroxy-4-pregnen-3-one, 17,20β-DP, MIS) [34]. In uterine homogenates of rat and human, 2-OHE_2 stimulated PGF production more than E_2 [25]. In rabbit preimplantation blastocyst and endometrial cells, catecholestrogens but not E_2 stimulated PG production [42]. The increased activity has been attributed to the presence of a catechol structure in 2-or 4-hydroxyestrogen, which confers increased chemical reactivity. Epinephrine with the catechol structure was moderately effective compared to 2-OHE_2 [25]. Similarly, norepinepherine stimulated $PGF_{2\alpha}$ level in cultured bovine endometrial cells [49]. Although the involvement of catecholestrogens in ovarian steroidogenesis, and FOM and ovulation has been demonstrated in the catfish [34,35] there are no studies to date on the role of catecholestrogens in the secretion of PGs.

In the present study, the role of 2-OHE_2 on ovarian PGE_2 and $PGF_{2\alpha}$ secretion was investigated and compared with that of E_2 and 2-methoxyE_2. In order to demonstrate the possible receptor mechanism, effects of exposure of the tissues to known inhibitors of estrogen (tamoxifen) and catecholamine (α- and β-adrenergic) receptors on PG production were compared with that of 2-OHE_2. Further, effects of 2-OHE_2 was evaluated in presence of inhibitors of the cell signaling pathways (cAMP-protein kinase A, PKC and mitogen-activated protein kinase (MAP kinase) to identify the pathways mediating the catecholestrogen action. For basic information, seasonal profile and periovulatory changes in both PGE_2 and $PGF_{2\alpha}$ were also demonstrated.

2. Materials and methods

2.1. Animal collection and acclimatization

Adult female *H. fossilis* (40–60 g) were purchased from local fish markets in Varanasi in June (late prespawning phase) and first week of July (spawning phase). They were maintained in the laboratory for 48 h under natural photoperiod and temperature to overcome stress due to transportation and fed daily with goat liver *ad libitum*. For seasonal study, fish were collected in different phases of the reproductive cycle, as mentioned below. The experiments were performed in accordance with local/national guidelines for experimentation in animals and all care were taken to prevent cruelty of any kind.

2.2. Chemicals

1, 3, 5 (10) Estratriene-3, 17β-diol (estradiol-17β, E_2 No. E1024), 1, 3, 5, (10) estratriene-2, 3, 17β-triol (2-hydroxyestradiol-17β, 2-OHE_2, No. H3131), 1, 3, 5 (10)-estratrien-2,3,17β-triol-2-methylether (2-methoxyE_2, No. M6383), 17,20β-dihydroxy-4-pregnen-3-one (17, 20β-DP. No. P6285), tamoxifen (No. T5648), 3-isobutyl-1-methylxanthine (IBMX, No. T5879), H89 dihydrochloride hydrate (H89, No. B1427), chelerythrine (No. C2932), PD098059 (No. P2157), prostaglandin $F_2\alpha$ (No. P0314), prostaglandin E_2 (No. P5640), phentolamine hydrochloride (PA, No. P7547), and propranolol (PP, No. P8688) were purchased from Sigma (St. Louis, MO, USA). Sucrose, sodium carbonate, sodium hydroxide, copper sulphate, monobasic sodium phosphate, dibasic sodium phosphate, methanol (HPLC grade), acetonitrile (HPLC grade) and ethyl acetate, orthophosphoric acid and other chemicals (analytical grade) were purchased from E. Merck, New Delhi, India. Human chorionic gonadotropin (hCG, Corion®, IBSA, Switzerland) was purchased from a local medical store. Degassed and filtered nanopure water (Bernstead, USA) was used throughout chromatography.

2.3. Preparation of incubation medium and test compounds

The incubation medium was prepared as follows: NaCl 3·74, KCl 0·32, $CaCl_2$ 0.16, NaH_2PO_4. H_2O 0.10, $MgSO_4 \cdot 7H_2O$ 0.16 and glucose 0.40 (all in g) were dissolved in 1 L of triple distilled water. The pH was adjusted to 7.5 with 1 M sodium bicarbonate and autoclaved. Penicillin (200,000 IU) and streptomycin sulphate (200 mg) were added and filtered. The medium was stored at 4 °C and prepared fresh every week. $PGF_{2\alpha}$ was dissolved in HPLC grade water and PGE_2 in HPLC grade ethanol to make stock solutions. Stock solutions of all steroids used in this study were made in HPLC grade methanol and diluted with the incubation medium just before incubation. The pH was readjusted to 7.4. Propranolol stock solution was prepared in culture medium while phentolamine was prepared in ethanol and further diluted with the culture medium.

2.4. Seasonal study of ovarian prostaglandins

In preparatory (March), prespawning (May), spawning (July), postspawning (October) and resting (December) phases, adult catfish weighing 40–60 g were purchased from a local fish market in Varanasi. Five fish each from the respective reproductive phases were sacrificed by decapitation. Ovaries ($n = 5$) were removed carefully, weighed and placed on ice. The ovarian pieces were processed for extraction and quantification of prostaglandins. Gonado-somatic index (GSI) was calculated from weight of the ovary expressed in 100 g body weight.

2.5. Effect of hCG on ovarian prostaglandin levels

2.5.1. In vivo study

In the spawning phase (first week of July), sexually mature female catfish were collected and acclimatized for 48 h. A few fish were randomly selected and ovaries were dissected out to check the stage of ovarian development. The ovaries contained gravid green eggs (~1 mm diameter). Forty acclimatized fish were divided into two groups of 20 each. The first group was injected with 100 IU/fish of hCG intraperitoneally. The second group was injected with an equal volume of vehicle (0.7% NaCl). Five fish each from the two groups were sacrificed by decapitation at 0, 8 and 16 h after the injection. At 16 h, the remaining fish in the two groups were hand-stripped for egg collection, and maintained up to 24 h until sampled. The ovaries were carefully removed on ice, weighed and processed for PG extraction and quantification by HPLC.

2.5.2. In vitro study

In late prespawning phase (last week of June), about 200 mg ovary pieces containing postvitellogenic follicles from each fish ($n = 3$) in triplicate were incubated with 5 ml of incubation medium containing 1, 5 or 10 IU hCG/mL for 0, 8, 16 and 24 h. Control groups (plain incubation medium containing the vehicle) were run parallel. After each 4 h, the incubation medium was changed and collected, and replenished with fresh medium containing the respective hormone concentration. After the completion of the