ROLE OF CATECHOLESTROGENS ON IN VITRO PROSTAGLANDIN SECRETION IN OVARIAN FOLLICLES OF THE CATFISH *HETEROPNEUSTES FOSSILIS*

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Introduction:

In teleosts, prostaglandins (PGs) have been implicated with oocyte final maturation, steroidogenesis and sexual behaviors. Its synthesis is modulated by gonadotropin, progestins or other steroids [1]. Catecholestrogens are estrogen metabolites, which have been implicated in reproductive functions such gonadotropin release, ovarian follicular steroidogenesis, prostaglandin synthesis, parturition, and embryo implantation [2]. Recently, catecholestrogens and estrogen-hydroxylases were characterized in the Indian catfish ovary with seasonal and periovulatory changes [3, 4]. Hydroxyestrogens have been shown to stimulate final oocyte maturation and ovulation by stimulating the secretion of the maturation-inducing steroids 17, 20β-dihydroxy-pregne-3-one production [5]. A direct role of catecholestrogens on PG production was demonstrated in higher animals, 2-hydroxyE_2 stimulated PGF production more than E_2 [6]. Present study demonstrates the role of 2-hydroxyE_2 on ovarian PG secretion in the catfish. Additionally, the pattern of secretion of the two PGs (PGE_2 and $PGF_{2\alpha}$) was monitored during the annual reproductive cycle of the catfish and during hCG-induced oocyte final maturation and ovulation.

Methods:

Five adult female fish (40-50g) each from preparatory, prespawning, spawning, postspawning and resting phases were sacrificed and ovaries were sampled. In the spawning phase, catfish (40-60g) was injected with 100 IU hCG/fish intraperitoneally or with an equal volume of vehicle (0.7 % NaCl) and sampled at 0, 8, 16 and 24 hr post injection for extraction and quantification of prostaglandins. In the prespawning phase, about 200 mg ovary pieces in triplicate from each fish (n = 3) were incubated with 5 ml of incubation medium containing 1, 5 or 10 IU hCG/ml or 1, 10, 100 and 1000nM of E_2 or catecholestrogens (2-hydroxyE_2 and 2-methoxyE_2) for 0, 8, 16 and 24 hr. In the second study, ovary pieces were incubated with 5 ml medium with or without 10nM, 100nM or 1μM each of phentolamine (α-adrenoceptor blocker), propranolol (β-adrenoceptor blocker), or tamoxifen (estrogen receptor blocker) alone or in combination with 1μM 2-hydroxyE_2. Control groups (plain incubation medium containing the vehicle) were run in parallel. After each 4 hr, the incubation medium was changed and collected, and replenished with fresh medium containing the respective hormone concentrations. The tissue and media were processed for extraction of PGs using a solid phase extraction method and quantified by HPLC method, as described by Jayadeep et al [7].

Results and Discussion:

The levels of both PGE_2 and $PGF_{2\alpha}$ increased significantly during ovarian recrudescence and peaked in the spawning phase. In vivo and in vitro, hCG stimulated PG production with peak secretion at 16 hr coinciding with oocyte final maturation and ovulation. The catfish ovary possesses the ability to convert estrogens into hydroxyestrogens and further into methoxyestrogens [3]. A concentration- or duration-dependent increase in PGs was noticed when follicles were incubated with E_2, 2-hydroxyE_2 and 2-methoxyE_2. 2-hydroxyE_2 was more potent than the other steroids. Phentolamine and propranolol (α-and β-antagonists, respectively) did not produce any significant change on basal PG levels but the incubation with tamoxifen lowered the PG levels. The pre-incubation of the follicles with tamoxifen, phentolamine and propranolol resulted in the inhibition of the stimulatory effect of 2-hydroxyE_2 on PGE_2 and $PGF_{2\alpha}$. Phentolamine (α-adrenergic blocker) was more effective, followed by tamoxifen and propranolol (β-adrenergic blocker) in inhibiting the 2-hydroxyE_2 effect.

Conclusion:

In conclusion both $PGF_{2\alpha}$ and PGE_2 showed significant seasonal variation and periovulatory changes. Catecholestrogens increased the PG secretion more than E_2. The 2-hydroxyE_2-induced stimulation appeared to be modulated through estrogen and catecholamine receptors.

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