

EFFECTS OF α -METHYLPARATYROSINE, A CATECHOLAMINE INHIBITOR ON OVARIAN STEROID HORMONE LEVELS IN THE CATFISH *HETEROPNEUSTES FOSSILIS*

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Introduction:

In higher vertebrates like mammals, the ovary elicits high catecholaminergic activity that originates either from extrinsic sympathetic innervation or intrinsic catecholaminergic system [1]. Ovarian innervation by the autonomic nervous system (ANS) has been described in a few teleosts and the innervations pattern shows considerable species variation [2]. Recent, investigation from our laboratory has demonstrated that the catfish ovary is innervated by seven pairs of nerves, originating from the paired sympathetic chain lying dorsal to the posterior kidney. Further we demonstrated tyrosine hydroxylase (TH, the rate limiting enzyme in catecholamine synthesis) and catecholamine seasonal activities in the ovary of the catfish during the annual reproductive cycle [3, 4] implying a functional role in ovarian gametogenesis and ovulation. Previous studies have shown that steroid hormone secretion is regulated by catecholamines [5]. However, investigations on the direct role of catecholamines in regulating ovarian steroid hormone levels are lacking. Since surgical denervation of the ovary, like in mammals is difficult. A pharmacological blocker to inhibit catecholaminergic activity was employed to investigate the role of catecholamines in ovarian function. The methylated derivative of tyrosine, α -methyl paratyrosine (α -MPT) is a competitive inhibitor of TH. This drug has been widely used as a pharmacological tool to investigate the CA metabolism [6]. In the present study, post vitellogenic follicles were incubated in vitro with α -MPT and steroid hormone levels were measured.

Materials and Methods:

The study was conducted during preparatory (March) and pre spawning (June) phases. The acclimatized fish were sampled for the collection of ovaries, weighed and transferred into a sterile petri dish containing freshly cooled incubation medium. The ovary pieces (about 350 mg each) were rinsed and transferred to culture plates containing 5ml medium each with α -MPT (250 μ g/ml), L-DOPA (1 μ g/ml) and hCG (20IU/ml) alone or in co-incubation. In co-incubation groups, ovaries were preincubated with α -MPT (250 μ g/ml) for 3 hr and then co-incubated with L-DOPA (1 μ g/ml), hCG (20IU/ml) or L-DOPA+ hCG (1 μ g/ml+ 20IU/ml) at 22^oC for 12 or 24 hr. All incubations were done in triplicate. The medium was changed after every 4 hr and collected group-wise.

After completion of the incubation, the tissues along with the incubation medium were collected separately and processed for steroid measurement by HPLC/ELISA, as described by Singh and Joy, [7]. Control groups (plain medium and medium containing vehicle) were set up in parallel. Data were presented as mean \pm SEM and analysed by two-way ANOVA, followed by Newman Keuls' test.

Results and Discussion:

In vitro incubations of ovarian pieces with α -MPT, L-DOPA and hCG, alone or in co-incubation, produced an overall significant effect on ovarian steroids. In the preparatory phase, the incubation with α -MPT decreased significantly both E₂ and P₄ in a time-dependent manner. The incubations with L-DOPA and hCG significantly increased both E₂ and P₄ in a duration- dependent manner, and the effect was higher in the hCG group. In co-incubation studies (α -MPT + L-DOPA, α -MPT + hCG and α -MPT+L-DOPA + hCG), L-DOPA or hCG reversed the inhibitory effect of α -MPT and restored the levels to that of the control or even higher but lower than that of L-DOPA or hCG per se. In the preparatory phase 17-P and 17, 20 β - dihydroxy-progesterone were not detectable. The incubations with L-DOPA and hCG decreased E₂ significantly, the inhibition was higher at 24 hr. The co-incubations with α -MPT reduced significantly the inhibitory effect of L-DOPA or hCG, and the response was higher in the 12 hr groups. α -MPT decreased P₄, 17-P and 17, 20 β -dihydroxy-progesterone levels in a time-dependent manner. The incubations with L-DOPA and hCG increased significantly P₄, 17-P and 17, 20 β -DP in a time- dependent manner. In the co-incubation groups (α -MPT + L-DOPA, α -MPT + hCG and α -MPT + L-DOPA + hCG), the inhibitory effect of α -MPT was reversed and progestin levels increased significantly but lower than that of the L-DOPA and hCG groups. The increase was higher in the α -MPT + L-DOPA + hCG group.

Conclusion:

Our investigation suggests that α -MPT and L-DOPA modulated in vitro ovarian steroid hormone production, influencing gametogenesis and ovulation and maturation of oocytes.

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