EFFECTS OF PERFUSION OF TESTICULAR FRACTIONS ON BRAIN CATECHOLAMINES IN THE FEMALE CATFISH *HETEROPNEUSTES FOSSILIS*

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Introduction:

Fish have highly developed chemosensory and chemical signaling systems as they inhabit aquatic environment devoid of light but rich in dissolved compounds. Four major classes of chemicals have been identified as specific olfactory stimuli namely amino acids, sex steroids, bile acids or salts and prostaglandins. Pheromones are defined as substances which are secreted to the outside by an individual, in which they release specific reaction; for eg; a definite behavior or a developmental process in conspecifics[1]. In many teleost species, pheromones play an important role in various events of reproductionand spawning [7,4]. The gonads of teleosts secrete pheromonally active substances, most likely steroidal glucuronides, which can activate the neuroendocrine axis to release gonadotropin–II. In the African catfish, strong olfactory sensitivity to steroid conjugate fraction has been attributed to glucuronides synthesized by the seminal vesicle [3,2]. It was reported that the testicular homogenate or its, glucuronide fraction [6] and the seminal vesicle fluid or its glucuronide fraction [2] induced either ovulation or behavior effects in zebrafish and African catfish, respectively. The testis of the *Heteropneutes fossilis* seems to synthesize steroid glucuronides like the seminal vesicle and may be responsible for the olfactory sensitivity [5]. Sex steroids have been reported to alter hypothalamic aminergic metabolism and modify gonadotropin secretion. In the present study, female catfish were perfused with testicular fractions in olfactory organ intact and ablated groups and brain catecholamines were measured to study the relationship between steroidal pheromones and catecholamine secretion during spawning phase.

Materials and Methods:

Female catfish *Heteropneustes fossilis* were collected from local fish markets during spawning phase (July - August) and acclimatized in laboratory conditions for 48hr. After acclimatization, intact or olfactory organ-ablated fish were perfused with various testicular (pooled from 20 fish) fractions (crude homogenate, organic fraction, aqueous fraction, aqueous fraction after β glucuronidase treatment and organic fraction after β glucuronidase treatment) for 18hr in a static system at a flow rate of 4ml/hr, individually (n = 5 fish). Each of the fraction was diluted in 70ml dechlorinated water and taken in a common reservoir tank connected to the aquarium tanks containing sham control (intact) or anosmic fish placed individually and perfused continuously at the rate of 4ml/hr. Brain tissues were collected at 18 hr by decapitation and transferred into vials containing 0.2M perchloric acid under dark conditions. The brain samples were homogenized and centrifuged at 10,000 rpm for 15 min at 4ºC. The supernatant was collected separately, protected from light and directly used for catecholamines assay by HPLC (Wood and Hall, 2000). The data were expressed as means ± SEM and analysed by one way analysis of variance (ANOVA), followed by Newman-Keuls' test ($p< 0.05$).

Results and Discussion:

In the present study, effects of perfusion of testicular fractions viz., whole homogenate (fraction 1), dichloromethane extract (fraction 2), aqueous extract (fraction 3), deglucuronated aqueous extract (fraction 4) and deglucuronated organic (fraction 5) on brain contents of catecholamines (dopamine-DA, norepinephrine-NE and epinephrine-E) and metabolite (3, 4-DOPAC and Normetanephrine) in female catfish *Heteropneustes fossilis* were studied. The content of brain DA increased in the OE-ablated groups compared to the OE-intact fish. However, fraction 4 decreased the DA level in comparison to other fractions perfused in OE-intact group. The DOPAC level decreased significantly in all ablated groups except the homogenate perfused group. The decrease was higher in aqueous fraction group. The DA turnover index was low in the OE-intact group in comparison to the OE-ablated group. It is the lowest in fraction 2, 3 and 4. The perfusion with various testicular fractions produced significant effects on brain contents of NE and NME. NE content increased with fractions 2, 3, 4 and 5 significantly, though the levels did not vary significantly between the fractions (3 and 4). Ablation of the olfactory organ abolished the stimulatory effect of the perfusion with all the fractions. On the other hand, in the perfused fish, NME content was increased in fractions 1, 2 and 5 and decreased in fraction 3 and 4 in olfactory organ intact fish. In ablated groups there was either no effect or a decrease in NME content with all fractions. The NE turnover index was significantly higher OE-intact group with fraction 2, 3 and 4. The content of epinephrine showed significant

increase when perfused with fractions 1, 2, 3 and 4. However, fraction 5 perfusion did not alter the content significantly in the olfactory organ intact fish. Ablation of the olfactory organ abolished the stimulatory effect of the perfusion with the fractions. The present study signifies that the perfusion with testicular fractions elicited changes in brain catecholamine levels in both sham anosmic (OE-intact) and anosmic (OE-ablated) fish.The changes in the content of DA, DOPAC, NE, NME, E and its turnover index may be attributed to the olfactory organ –mediated pheromonal activity of testicular fractions since such changes were not significant in anosmic fish. The results strongly support the existence of a neuroendocrine pathway involving catecholaminergic system to mediate sex pheromone-mediated changes.

Conclusion:

In conclusion our investigation suggests that testicular pheromones, which on release into water medium may form a chemical communication link by interacting with the olfactory–neuroendocrine-gonadal axis of the conspecifics, by altering central catecholaminergic activity.

References:

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