



## EFFECTS OF ETIOCHOLANOLONE GLUCURONIDE, A PUTATIVE PHEROMONE ON BRAIN AND PLASMA VASOTOCIN LEVELS IN THE CATFISH *HETEROPNEUSTES FOSSILIS*

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### Introduction:

Teleost gonads have been demonstrated to be an active source of sex (reproductive) pheromones having releaser or primer functions. Steroid hormones, prostaglandins and their metabolites have received considerable attention in this regard. Etiocholanolone glucuronide (EG) is a natural metabolite of etiocholanolone generated in the liver by UDP gluconyl transferase. Etiocholanolone (or 5-isoandrosterone) is a metabolite of testosterone, classified as a ketosteroid; it causes fever, immunostimulation and leukocytosis. Glucuronidation is a means of excretion of toxic substances, drugs or other substances that cannot be used as an energy source. In teleosts, EG is secreted by males (testis and seminal vesicle) and exerts pheromonal roles via olfactory stimulation of females [1]. This steroid has got pheromonal properties and is responsible for regulation of spawning behavior and reproduction. Vasotocin (VT) is the basic neurohypophysial nonapeptide in nonmammalian vertebrates and is involved in diverse functions like osmoregulation, reproduction and behavior [2]. The administration of VT has been shown to influence or modify reproductive/seasonal behavioral activities (spawning, courtship, egg laying, clasping and song production) in a variety of vertebrates like fish, amphibians, reptiles and birds [3, 4, 5]. The reproductive role of VT was investigated in the catfish and ovarian steroids were found to modulate VT secretion in vitro [6]. We, therefore, hypothesize that pheromone exposure may

induce brain VT release and influence catfish reproductive behavior. To test this hypothesis we perfused female catfish with different concentrations of EG and measured brain and plasma vasotocin levels.

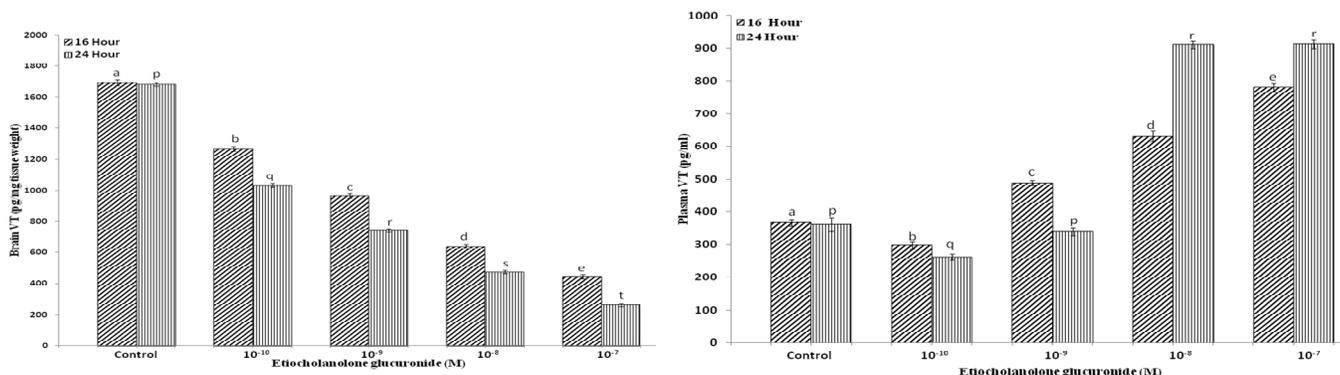
### Methods:

Female catfish *Heteropneustes fossilis* were collected from local fish markets during spawning phase (June - July) and acclimatized in laboratory conditions for 48hr. After acclimatization, intact or olfactory organ-ablated fish were perfused with different concentrations of EG ( $10^{-7}$ ,  $10^{-8}$ ,  $10^{-9}$  and  $10^{-10}$  M) for 16 or 24 hr in a static system at a flow rate of 4ml/hr, individually (n = 5 fish). EG was dissolved in 100µl of methanol and then final concentrations were made in fish saline. After blood plasma collection, the fish were sacrificed by decapitation and brains along with pituitary were removed and dropped into cold anhydrous acetone and stored at -70°C. Brains and plasma were processed for VT extraction and quantification by EIA kit (Bachem Peninsula Laboratories, California, USA; Catalogue No. S - 1239, EIAH - 8121). Data were presented as mean ± SEM and analyzed by one way ANOVA, followed by Tukeys' test.

### Results and Discussion:

The perfusion of the olfactory organ-intact fish with different concentrations of EG produced an overall significant effect on brain and plasma VT levels (Fig. 1). EG decreased brain VT levels significantly in a concentration-dependent manner in comparison to the

**Fig. 1.** Effects of perfusion of different concentrations of etiocholanolone glucuronide on brain (A) and plasma (B) VT levels during spawning phase





control group but increased plasma VT levels at both 16 and 24 hr. In the olfactory organ-ablated fish, there was no significant effect compared to the control group. The results suggest that EG perfusion increases the release of VT from the pituitary to the circulation causing a decline in concentration in the brain and an increase in the plasma. Thus, pheromonal stimulation can regulate VT secretion and influence reproductive behavior in the catfish. The pheromonal chemosensory system can transmit social stimuli between conspecific individuals that can induce reproductive behavior in the recipients, in this case females. VT has been shown to stimulate the synthesis of the MIS and induce ovulation in the catfish at ovarian level. It has been reported that sensory organs are the filters through which organisms determine the context of their environment and respond accordingly. Pheromones have diverse roles in modulating vertebrate behavior [7]. Social stimuli often evoke stereotypical behavioural responses in conspecifics, potentially by activating endogenous neuropeptide systems, notably those related to vasotocin (VT) and its mammalian derivative, vasopressin (VP). These peptides influence a variety of species-specific social behaviors across vertebrates [8] and social stimuli can activate VT/VP cells and drive release of the peptides in the brains of at least some species.

#### Conclusion:

In conclusion, the present study demonstrates that EG, as a putative pheromone can regulate VT function, thereby, influencing reproductive behavior and spawning activity in the catfish. This is the first report correlating functional interplay between pheromonal and VT systems.

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