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Estrogen regulation of brain vasotocin secretion in the catfish *Heteropneustes fossilis*: An interaction with catecholaminergic system

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ABSTRACT

Vasotocin (VT) is a basic neurohypophysial nonapeptide in non-mammalian vertebrates and is involved in diverse functions like osmoregulation, reproduction, metabolism and behavior. In this study, we report that estradiol-17β (E_2) regulates brain and plasma VT secretion through the involvement of the catecholaminergic (CA) system. To demonstrate this, E_2 level was altered through ovariectomy (OVX, 3 weeks) and replacement study with low and high E_2 doses (0.1 and 0.5 μg/g body weight). CA activity was inhibited by treatment with α-methylparatyrosine (α-MPT; 250 μg/g body weight), a competitive inhibitor of tyrosine hydroxylase. VT was assayed by an enzyme immunoassay method. In the sham group, the low E_2 dose produced 82% and 104% increase, respectively, in brain and plasma VT levels. The high E_2 dose decreased the VT levels significantly. The low E_2 dose decreased brain E_2 but elevated plasma E_2. In the high E_2 group, the E_2 level increased further in both brain and plasma. OVX resulted in a significant inhibition (69% and 25%, respectively) of both brain and plasma VT, which was correlated with low E_2 levels. The low E_2 dose not only reversed the inhibition, but increased the VT level in both brain and plasma in comparison to the sham groups. The high E_2 replacement inhibited VT levels further low in both brain and plasma. The α-MPT treatment inhibited VT levels significantly in both sham and OVX groups. The drug treatment abolished partially the restorative effect of the low E_2 dose in the ovariectomized fish. In the high E_2 dose group, α-MPT decreased brain and plasma VT levels further low compared to the sham + 0.5 μg E_2 group or OVX + 0.5 μg E_2 group except the brain VT level, which increased in the OVX + 0.5 μg E_2 group. It is inferred that E_2 may exert biphasic effects on VT through the mediation of the CA system.

1. Introduction

Vasotocin (VT) is a basic neurohypophysial nonapeptide in non-mammalian vertebrates and the evolutionary predecessor of vasopressin [2,20]. The peptide is synthesized in the magnocellular neurons of the hypothalamus, transported and stored in the neural lobe, and released into the circulation for physiological action such as water balance, metabolism, reproduction and behavior [6]. VT is diuretic in freshwater fishes but antidiuretic at low doses, and antidiuretic in non-mammalian tetrapods [6,34,51]. A reproductive role of VT such as oviduct and uterine contraction, courtship or spawning behavior, oviposition and parturition has been demonstrated in fishes, amphibians, reptiles and birds [17,24,26,37,46,50,54,65]. VT is present in peripheral tissues outside the nervous system in birds, amphibians and fishes [18,38]. In the catfish, the presence of VT was demonstrated in the follicular layer of the ovary [56] where it is involved in steroidogenesis, final oocyte maturation and ovulation [57–59].

In mammals, vasopressin (VP) is regulated by a variety of neurotransmitters and neuropeptides such as acetylcholine, catecholamines, serotonin, gamma-aminobutyric acid, glutamate, nitric oxide and angiotensin-II [9]. An anatomical proximity of the monoamine- and neurophysin-containing pathways has been demonstrated, implying their functional interaction [35]. Catecholaminergic neurons make synaptic contacts with VP-ergic neurons in the hypothalamus [55] and all three types of adrenoceptors (α_1, α_2, and β) are present on supraoptic and paraventricular nuclei [62]. The α_1 mediates excitatory signals, while the other two types elicit inhibitory signals [9]. Dopamine has been reported to stimulate or inhibit VP secretion through D_1 or D_2 receptors [9,19,48]. However, the neural regulation of VT secretion has not been investigated in lower vertebrates.

Gonadal steroid hormones have been reported to modulate the secretion of neurohypophysial nonapeptides in different vertebrates [38,39]. In salmon changes in brain VT and isotocin (IT) mRNA levels were correlated with estradiol and testosterone levels

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[42,43]. In the catfish estradiol-17β (E_2) administration modulated brain and ovarian VT secretion; low doses stimulated, and high doses inhibited VT levels [58]. In bull frog gonadectomy decreased VT content in brain regions and estradiol treatment fully restored it in certain brain areas [10]. In cycling female rats, pituitary levels of oxytocin (OT) and VP were maximal on the morning of proestrus, declined during estrus, and were lowest on metestrus [15]. In cows ovarian OT and VP levels varied during the estrous cycle, the highest levels were found in the first half of the luteal phase [66]. A functional correlation between gonadal steroids, and plasma VP and OT was implicated through numerous studies involving gonadectomy and steroid replacement experiments [3,16,60,61]. Gonadal steroid hormones and neurohypophysial octapeptides interact to regulate reproductive behavior in mammals as well as in non-mammalian vertebrates [10,44,45,47,49]. Sex steroids induce organizational changes on neurons, causing an increase or change in cell body size or shape, changes in neuronal enzyme content, neuropeptide and neurotransmitter production, growth of dendrite processes, and modification or alteration of efferent and afferent circuits [44,49]. Sex steroids can alter expression of genes encoding for VP, VT or OT [41,63]. While anatomical, physiological and molecular studies strongly indicate functional interaction between neural and gonadal steroid hormone control of VP secretion in mammals, such information on VT control is lacking in fishes.

In the present study, we report the effects of ovariectomy and E_2 replacement, and α-MPT treatment in both sham and ovariectomized/E_2 replaced fish on VT secretion in order to elucidate the possible interaction between E_2 feedback and catecholaminergic system on the regulation of VT secretion.

2. Materials and methods

2.1. Animal collection and acclimatization

The experiments were performed in accordance with local/national guidelines for experimentation in animals and care was taken to prevent cruelty of any kind. Live sexually mature female catfish (40–50 g) were collected from local fish markets in the first week of May (prespawning phase) when the ovaries were in post-vitellogenic phase. Our previous study showed that the circulating levels of both VT and E_2 are high during this period. They were maintained in flow – through aquarium tanks under normal photoperiod and ambient temperature (13:21 h L: 10:39 h D and 24 ± 2 °C) for 48 h before sampling. During the acclimatization, the fish were fed daily minced goat liver *ad libitum*.

2.2. Chemicals

Estradiol-17β (E_2), α-methylparatyrosine (α-MPT) and 3-aminobenzoic acid ethylester (MS222) were purchased from Sigma Chemical Company, St. Louis, USA. (Arg^8) – Vasotocin enzyme immunoassay kit [(EIA kit, Catalogue No. S-1239) (EIAH 8121)] was purchased from Bachem Peninsula Laboratories, California, USA. Solid phase extraction (SPE) C18 cartridges were purchased from Ranbaxy Fine Chemicals Ltd., Ghaziabad, India. Other chemicals used were of analytical grade and purchased from E. Merck, Mumbai, India. Degassed and filtered nanopure water (Barnstead International, Dubuque, IO, USA) was used throughout ELISA.

2.3. Experiments

2.3.1. Ovariectomy and E_2 replacement

Fish were ovariectomized or sham ovariectomized in the prespawning phase, as described earlier [52]. The operated fish were maintained for 3 weeks. Mortality was up to 3%. Completeness of ovariectomy and regeneration of gonads, if any, were checked by examining the peritoneal cavity of the fish at the time of sampling. Only tissues from completely ovariectomized fish were used for the study. For E_2 replacement, low (0.1 μg/g body weight, BW) and high (0.5 μg/g BW) doses were selected, as used previously in our laboratory [53]. E_2 was injected intraperitoneally daily for 3 days in 3-week ovariectomized and sham ovariectomized fish ($n = 5$). As vehicle control, five fish each from the ovariectomized and sham ovariectomized groups were given an equal volume (100 μL) of vehicle (propylene glycol).

2.3.2. α-MPT treatment

Fish were ovariectomized and sham ovariectomized, grouped and E_2 was injected, as described above. For inhibition of catecholamine activity, α-methylparatyrosine (α-MPT), a competitive inhibitor of tyrosine hydroxylase was injected intraperitoneally (250 μg/g BW) daily for 3 days, in sham ovariectomized, ovariectomized, and ovariectomized-E_2 treated groups. α-MPT was dissolved in half the required volume of 0.65% NaCl (fish saline) at pH 10 with 5 N NaOH, rapidly precipitated by acidifying to pH 1.5 with 5 N HCl and diluted with the remaining amount of saline to give a final pH 7.8 [15]. The dose was selected based on earlier studies [12,53]. As control, five fish each were given an equal volume of propylene glycol or α-MPT vehicle.

All fish were sampled between 0900 and 1100 h and the peritoneal cavity was examined for any regeneration of ovaries, after blood collection. Blood samples were drawn from caudal vein in heparinised vials and were kept at 4 °C for separation of plasma. The samples were centrifuged at 2800*g* at 4 °C for 15 min and plasma was collected in separate tubes and stored at −70 °C, until the assay. After blood collection, the fish were sacrificed by decapitation and the brains along with pituitaries were removed, dropped into cold anhydrous acetone and stored at −70 °C. The peptide was extracted from the samples within a week of the storage.

2.4. E_2 extraction and assay

The tissues were homogenized separately or group-wise in 4 volume of cold PBS (0.02 M, Phosphate buffer saline, pH 7.4) with an ultrasonic homogenizer (XL – 2000 Microson, Misonix, USA) at 0 °C for 5–10 s. The homogenate was centrifuged at 5000*g* for 20 min at 4 °C and extracted with 3 volume of diethyl ether, three times. The ether phase was collected, pooled, evaporated and dried under N_2 gas and stored at −20 °C till further processed for steroid estimation. E_2 was assayed using an ELISA kit (Diametra, Italy) according to the manufacturer's instructions. Briefly 25 μL each of the standard (0, 20, 120, 300, 600 and 2000 pg/mL) and samples were pipetted into the antiE_2-IgG-coated plate wells. The immunoreaction was started by adding 100 μL of E_2–HRP conjugate solution to each well followed by incubation at 37 °C for 2 h. The content from each plate was removed and washed with 300 μL of distilled water, 5–6 times. Water was completely drained out from each well. Next 100 μL of 3, 3′, 5, 5′-tetramethylbenzidine (TMB) substrate was dispensed into each well and incubated at 25 °C for 30 min in dark. Color development was stopped by adding 100 μL of stop solution (0.15 mol/L sulfuric acid). Absorbance was taken at 450 nm using a Multiscan EX (Thermo Lab system) ELISA reader. The standard curve was plotted and readings were taken with the help of the software. The assay was validated previously in our lab [36].

2.5. Vasotocin extraction and assay

The peptide was extracted from the samples by reverse phase chromatography, as described by Singh and Joy [59]. Brain and plasma samples were quantified by an EIA method (Bachem Peninsula