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Differential and reproductive stage-dependent regulation of vasotocin secretion by catecholamines in the catfish *Heteropneustes fossilis*

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ABSTRACT

Vasotocin (VT) is the basic nonapeptide hormone secreted by the neurohypophysis of non-mammalian vertebrates and is involved in the regulation of osmoregulation, metabolism, cardiovascular function, reproduction and behaviour. Among the reproductive function, VT is specifically implicated in final oocyte maturation, ovulation, oviposition/parturition in teleosts, amphibians, reptiles, and birds. The central catecholaminergic system is involved in the regulation of pituitary hormone secretion including gonadotropin, and mediates also changes in environmental photoperiod and temperature. The close apposition of the VT and catecholaminergic systems in the preoptic area of the hypothalamus signifies a strong possibility of their functional interaction. The aim of the present study was to investigate the effects of exogenously administered catecholamines on VT secretion in two different reproductive phases of female catfish *Heteropneustes fossilis*. For this, the catecholamine precursor L-dihydroxyphenylalanine (L-DOPA) and catecholamines (dopamine-DA, norepinephrine-NE, and epinephrine-E) were intraperitoneally injected in normal catfish and/or along with α-methylparatyrosine (α-MPT, a tyrosine hydroxylase inhibitor). Brain and plasma VT levels were measured by specific ELISA, 24 h post injection. Both L-DOPA and DA inhibited brain and plasma VT levels in a concentration-dependent manner in preparatory and prespawning phases. In contrast, NE elicited dose-dependent effects: the lowest dose (0.5 ng/g body mass, BM) was ineffective, the median dose (1 ng/g BM) stimulated, and the high doses (10 and 100 ng/g BM) inhibited VT levels. E stimulated VT levels dose-dependently. A single injection of α-MPT (250 μg/g BM) strongly inhibited VT when given alone and enhanced the inhibitory effects of L-DOPA and DA in the combination groups. The α-MPT inhibition of VT was significantly reduced by the injection of NE (5 ng/g BM) and was restored or elevated by E. When the adrenergic neurotransmitters were given together with α-MPT, the inhibitory effect of the latter was abolished and VT levels were significantly elevated. Thus, the present data indicate that the physiological changes in VT is differentially regulated by the catecholamines (DA inhibits and NE/E stimulates VT).

1. Introduction

Vasotocin (VT) is the basic neurohypophysial nonapeptide hormone in non mammalian vertebrates and the evolutionary predecessor of vasopressin (VP) (Acher, 1996). In non-mammalian vertebrates, it is secreted by the magnocellular neurons of nucleus preopticus (NPO) or supraoptic and paraventricular nuclei (SON and PVN), along with the neutral nonapeptide isotocin (IT) or mesotocin (MT), the homologue of oxytocin (OT). VT is involved in various functions like water/salt homeostasis, metabolism, stress, reproduction, sexual and social behaviours (Balment et al., 2006; Goodson and Thompson, 2010). Apart from brain, VT is also distributed in peripheral organs like the ovary, uterus testis, adrenal and sympathetic system of higher vertebrates (Clements and Funder, 1986; Saito et al., 1990; Barth et al., 1997). Among the reproductive functions, VT is implicated in spawning behaviour, courtship, and oviposition/parturition (Pickford and Strecker, 1977; Guillette and Jones, 1982; Moore, 1992; Salek et al., 2002). In the catfish, VT is secreted by the NPO, axonally transported to the neurohypophysis and stored or released into the circulation for physiological activity. VT is also secreted ectopically in the gonads (Singh and Joy, 2008). VT showed significant seasonal variation in both brain and ovary with the levels increasing in the recrudescent phase and decreasing after spawning (Singh and Joy, 2008). In the ovary, it is secreted by the follicular layer and is involved in steroidogenesis, final oocyte maturation and ovulation (Singh and Joy, 2009a, 2009b). Thus, the peptide has a direct involvement in reproduction, like the gonadotropin.

Abbreviations: α-MPT, α-methylparatyrosine; BM, body mass; DA, dopamine; DOPA, L-dihydroxyphenylalanine; E, epinephrine; NE, norepinephrine; VT, vasotocin.

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The regulation of nonapeptide hormones in the vertebrates has been investigated in detail only in mammals with reference to VP and OT secretion (Bisset and Chowdrey, 1988; Gimpl and Fahrenholz, 2001). VP is regulated by a variety of neurotransmitters and neuropeptides such as acetylcholine, catecholamines, serotonin, gamma-aminobutyric acid, glutamate, nitric oxide and angiotensin-II. The SON/PVN receives catecholaminergic innervation that is largely noradrenergic but to a less extent dopaminergic (Decavel et al., 1987). Catecholaminergic (noradrenergic) neurons in the ventrolateral medulla make synaptic contacts with VP neurons in the hypothalamus (Shioda and Nakai, 1996). All three types of adrenergic receptors (α1, α2 and β) were described in the SON and PVN (Bisset and Chowdrey, 1988; Takano et al., 1989): α1 mediates excitatory signals and the other two elicit inhibitory signals. Dopamine exerts stimulatory, inhibitory or no role in VP secretion (Forsling and Williams, 1984; Reid et al., 1986; Bisset and Chowdrey, 1988; Rossi, 1998). However, studies on the regulation of VT secretion in non-mammalian vertebrates are few and limited to the role of gonadal steroids. Like in mammals (DeVries et al., 1985; Adan and Burbach, 1992; Sladek et al., 2000; Sladek and Somponpum, 2008), gonadal steroid hormones alter or modify VT secretion in fishes, amphibians, reptiles and birds (Moore et al., 1992; Boyd, 1994; Balthazart et al., 1996; Ota et al., 1996; Panzica et al., 1997; Moore and Lowry, 1998; Panzica et al., 1998; Ota et al., 1999; Singh and Joy, 2009a; Chaube et al., 2012). In a preliminary study Chaube et al. (2012) reported that α-methylparatyrosine (α-MPT), a tyrosine hydroxylase (TH) inhibitor, inhibited VT secretion and interacts with the estrogen feedback system, suggesting a catecholaminergic involvement, similar to VP secretion. That study, however, did not examine the specific role of different catecholamines on VT secretion.

Catecholamine (CA) neurotransmitters are crucial to the functioning of both central and peripheral nervous systems that regulate and coordinate a variety of functions such as motor activity, neuroendocrine regulation, biorhythms, feeding, mating behaviour, emotion, learning and memory, and peripheral sympathetic functions (Melmon, 1982; Nagatsu, 1991). The critical role of CAs in the regulation of pituitary hormones directly or indirectly by modifying hypothalamic neurohormones has been studied in depth in mammals (Melmon, 1982) and a common pattern is seen in all sub-mammalian vertebrates (Trudeau et al., 1993; Joy, 1999). In teleosts, CAs play an important role in the regulation of gonadotropin secretion both at pituitary and brain levels (Schulz et al., 1995; Peter and Yu, 1997; Joy, 1999) and mediate both photoperiod-temperature and estrogen feedback effects on the brain-pituitary ovarian axis (Senthilkumaran and Joy, 1995, 1996; Chaube and Joy, 2003).

The preoptic area that contains the neuroendocrine centres like the NPO, nucleus preopticus periventricularis (NPPv) is the regulatory centre controlling reproduction and sexual behaviour and sites of the estrogen feedback, catecholaminergic activity, and VT synthesis (Cerdá-Reverter and Canosa, 2009; Kah, 2009; Kawabata et al., 2012). Neuroanatomical evidence for interactions among DA-, estrogen/aromatase- and nonapeptide-containing neuronal systems in the preoptic area is presented previously (Kah et al., 1986; Joy et al., 1992; Marsh et al., 2006; Maruska, 2009; Kawabata et al., 2012; Singh et al., 2012). In the catfish *Clarias batrachus*, Singh et al. (2012) have shown that DA neurons in the NPP anterior and NPP posterior innervate IT neurons in the NPO. In the catfish, VT and TH show sex differences and are targets of estrogen feedback (Chaube and Joy, 2003; Singh and Joy, 2008, 2009a, 2009b).

Given the limited information on the functional interaction of the CA and VT systems in the lower vertebrates, the present study was undertaken in the catfish with the objectives 1) to demonstrate specific and differential effects of CAs, 2) whether the pharmacological impairment of the CA system by α-MPT can be corrected by CA treatments, and 3) whether the effects of the CAs are reproductive stage-specific. We present evidence for differential and reproductive stage-specific effects of the exogenously administered CAs on VT secretion.

2. Materials and methods

2.1. Animal collection and acclimatization

Live adult sexually mature female catfish (*Heteropneustes fossilis*, 40–50 g) of the first sexual cycle were collected from a local fish market in Varanasi. The experiments were conducted in preparatory phase [early vitellogenic ovary with gonado-somatic index (GSI) — 0.62%, the last week of March and third week of April 2010] and in prespawning phase, late vitellogenic ovary with GSI — 8.35%, last week of May and first week of June 2010. They were maintained in flow-through aquarium tanks under normal photoperiod and ambient temperature for 48 h before experiments. During acclimatization, the fish were fed daily with minced goat liver ad libitum. The experiments were performed in accordance with local/national guidelines for experimentation in animals.

2.2. Chemicals

α-Methylparatyrosine (α-MPT), dopamine (DA), L-3,4-dihydroxyphenylalanine (L-DOPA), norepinephrine (NE), epinephrine (E) and 3-aminobenzoic acid ethyl ester (MS222) were purchased from Sigma-Aldrich Chemical Company, St. Louis, MO, USA. (Arg^8)-vasotocin enzyme immunoassay kit [(EIA kit, catalogue no. S-1239) (EIAH 8121)] was purchased from Bachem Peninsula Laboratories, CA, USA. Solid phase extraction (SPE) C18 cartridge was 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, IA, USA) was used throughout ELISA.

2.3. Preparation of drug solution

α-Methylparatyrosine was dissolved in half the required volume of 0.65% NaCl (fish saline) at pH 10 with 5 N NaOH and 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 (Chaube and Joy, 2003). L-DOPA was dissolved in 0.2 M perchloric acid (PCA) and made up to the desired volume with triple distilled water (TDW). DA, NE and E were dissolved in TDW. All injections were given between 9:00–9:30 h.

2.4. Experiments

2.4.1. L-DOPA treatment

In the preparatory and prespawning phases, 30 acclimatized catfish were divided into 6 groups of 5 each. Group 1 was untreated control. Group 2 was injected with normal saline (0.1 mL, 0.65% NaCl, w/v). Groups 3–6 each were given a single i.p. injection of L-DOPA in doses of 0.5, 1.0, 10.0 and 100 ng/g body mass (BM), respectively.

2.4.2. Catecholamine (DA, NE and E) and α-MPT treatments

In the preparatory and prespawning phases, 120 acclimatized catfish each were divided into 4 groups of 30 each. The fish in each group were divided into 6 subgroups of 5 each. Subgroup 1 was untreated control. Subgroup 2 was injected with vehicle. Subgroups 3–6 each were given a single intraperitoneal (i.p.) injection of DA, NE or E in doses of 0.5, 1.0, 10.0 and 100 ng/g BM, respectively. In the remaining group, fish were injected with α-MPT in doses of 25, 50,100 and 250 μg/g BM.

2.4.3. α-MPT, L-DOPA and DA treatments

In both phases, a group of 40 acclimatized catfish were divided into 8 groups of 5 each. Group 1 was untreated control. Group 2 was injected with vehicle. Groups 3, 4 and 5 each were given a single i.p. injection of α-MPT (250 μg/g BM), L-DOPA (10.0 ng/g BM) or DA (10.0 ng/g BM), respectively. Groups 6, 7 and 8 each were given a single i.p. injection