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Distribution of hypothalamic vasoactive intestinal peptide immunoreactive neurons in the male native Thai chicken

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ABSTRACT

Avian prolactin (PRL) secretion is under stimulatory control by the PRL-releasing factor (PRF), vasoactive intestinal peptide (VIP). The neuroendocrine regulation of the avian reproductive system has been extensively studied in females. However, there are limited data in males. The aim of this study was to elucidate the VIPergic system and its relationship to PRL and testosterone (T) in the male native Thai chicken. The distributions of VIP-immunoreactive (-ir) neurons and fibers were determined by immunohistochemistry. Changes in VIP-ir neurons within the nucleus inferioris hypothalami (IH) and nucleus infundibuli hypothalami (IN) areas were compared across the reproductive stages. Plasma levels of PRL and T were determined by enzyme-linked immunosorbent assay and then compared across the reproductive stages. The results revealed that the highest accumulations of VIP-ir neurons were concentrated only within the IH-IN, and VIP-ir neurons were not detected within other hypothalamic nuclei. Within the IH-IN, VIP-ir neurons were low in premature and aging males and markedly increased in mature males. Changes in VIP-ir neurons within the IH-IN were directly mirrored with changes in PRL and T levels across the reproductive stages. These results suggested that VIP neurons in the IH-IN play a regulatory role in year-round reproductive activity in males. The present study also provides additional evidence that VIP is the PRF in non-seasonal, continuously breeding equatorial species.

1. Introduction

Avian prolactin (PRL) secretion and its gene expression are tonically stimulated (Kragt and Meites, 1965; Bern and Nicoll, 1968) by vasoactive intestinal peptide (VIP), the avian PRL releasing factor (PRF), which is secreted from neurons located in the infundibular nuclear complex (INF) of the hypothalamus (Chaiseha and El Halawani, 2015). In female birds, VIP acts directly on the pituitary gland to stimulate PRL synthesis and secretion during the reproductive cycle (El Halawani et al., 1997). Immunocytochemical studies have reported that VIP-immunoreactive (-ir) neurons within the INF and VIP-ir fibers in the median eminence (ME) correspond to the enhanced circulating PRL levels

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in turkeys and native Thai chickens (Mauro et al., 1989; Kosonsiriluk et al., 2008). Increases in the number and size of VIP-ir neurons within this hypothalamic region have also reported in domesticated pigeons and ring doves during periods of hyper-prolactinemia (Peczely and Kiss, 1988; Cloues et al., 1990).

The distributions of VIP-containing neurons and fibers have been mapped in many avian species such as Pekin ducks (Korf and Fahrenkrug, 1984), bantams (Macnamee et al., 1986), pigeons (Hof et al., 1991), ring doves (Norgren and Silver, 1990), dark-eyed juncos (Saldanha et al., 1994), chicks (Kuenzel et al., 1997), turkeys (Chaiseha and El Halawani, 1999), Japanese quails (Teruyama and Beck, 2001), collared doves (Den Boer-Visser and Dubbeldam, 2002), starlings (Dawson et al., 2002), zebra finches (Kingsbury et al., 2015), blue tits (Montagnese et al., 2015), and native Thai chickens (Kosonsiriluk et al., 2008). In the native Thai chicken, VIP-ir neurons and fibers are extensively distributed throughout the brain and are predominantly expressed in the diencephalon, where VIP-ir neurons are concentrated within the nucleus inferioris hypothalami (IH) and nucleus infundibuli hypothalami (IN) areas. Changes in the number of VIP-ir neurons within the IH-IN are directly correlated to plasma PRL levels across the reproductive cycle (Kosonsiriluk et al., 2008), and the number of VIP-ir neurons decreases concurrently with circulating PRL levels in nest-deprived incubating and disruption of rearing behavior hens (Prakobsaeng et al., 2011; Chaiyachet et al., 2013b). These findings suggest that VIP expression in the IH-IN plays a regulatory role in year-round reproductive activity and indicate its importance in the regulation of reproductive activity in this species (Kosonsiriluk et al., 2008).

The native Thai chicken, an equatorial, tropical, non-seasonally breeding species, has been domesticated without genetic selection. It expresses strong maternal behaviors, which are inherited from the ancestor, the wild jungle fowl (Sawai et al., 2010). It is well established that the neuroendocrine regulation of maternal behaviors (incubation and rearing behaviors) in the female native Thai chickens are associated with the gonadotropin releasing hormoneergic (GnRHergic), VIPergic, dopaminergic (DAergic), and mesotocinergic (MTergic) systems (Prakobsaeng et al., 2011; Sartsoongnoen et al., 2012; Chaiyachet et al., 2013a,b; Chockchaloemwong et al., 2013, 2015). Recently, behavioral endocrine studies in galliform birds have focused on the roles of several neurotransmitters, neurohormones, and hormones that function in maternal care behaviors. As indicated, the neuroendocrine regulation of rearing behavior has been extensively studied, particularly in females. However, there are limited data regarding the neuroendocrine regulation of parental behaviors in males. Indeed, in many species, male birds show parental care behaviors such as nest building, brooding, and feeding of the young (Chaiseha and El Halawani, 2015; Lynn et al., 2015). These phenomenons involving parental behaviors may occur due to a complex neuronal/hormonal interaction of many hormones, neurohormones, neuromodulators, and neurotransmitters.

To date, there has been no report on the VIPergic system of the male native Thai chicken. The aim of this study was to delineate the hypothalamic VIPergic system in the male native Thai chicken and investigate its relationship to PRL and testosterone (T). The findings of the differential distribution of hypothalamic VIP neurons and fibers and their associated circulating levels of PRL and gonadal steroids may provide an insight into the role(s) of the VIP/PRL system in the neuroendocrine regulation of reproductive activities in male galliform birds.

2. Materials and methods

2.1. Experimental animals

Male native Thai chickens (*Gallus domesticus*), ranging between 5 and 36 months old and mature females, ranging between 20 and 24 weeks old, were used. They were reared and housed (8–10 females:1 male) in floor pens equipped with basket nests under natural light (approximately 12 h of light and 12 h of darkness; 12L:12D). Feed and water were given ad libitum. The animal protocols used adhered to the guidelines approved by the Suranaree University of Technology Animal Care and Use Committee.

2.2. Experimental procedures

2.2.1. Experiment 1: distribution and localization of VIP-ir neurons and fibers in the hypothalamus of the male native Thai chicken

To determine the distribution of VIP-ir neurons and fibers in the hypothalamus of the male native Thai chicken, 6 mature males (about 12 months old) were used. The brains were fixed by pressure perfusion with 4% paraformaldehyde prior to sectioning in a cryostat and further processing by immunohistochemistry (IHC).

2.2.2. Experiment 2: changes in number of VIP-ir neurons in the IH-IN area and plasma levels of PRL and T across the reproductive stages of the male native Thai chicken

To compare changes in the number of VIP-ir neurons within the IH-IN and plasma PRL and T levels, 21 male native Thai chickens, 5–36 months, were used. The males were divided into 3 groups (7 birds/group) according to their reproductive stages: Group 1, premature male, 6 months old; Group 2, mature male, 12 months old; and Group 3, aging male, 36 months old. Birds were sacrificed according to their reproductive stages. Blood samples were collected from the brachial vein of each bird. The blood samples were then fractionated by centrifugation, and the plasma was stored at −20 °C until assayed for PRL and T. The brains were fixed by pressure perfusion with 4% paraformaldehyde, sectioned with a cryostat, and processed by IHC. A postmortem examination of each male was performed to confirm its reproductive stage.

2.3. Measurement of plasma PRL levels

Plasma PRL levels were measured using an enzyme-linked immunosorbent assay (ELISA) according to a previously described method (Kosonsiriluk et al., 2008). The plasma PRL levels determined by this assay in native Thai chickens were validated using the parallelism test