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Neuroendocrine regulation of rearing behavior in the native Thai hen

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ABSTRACT

Vasoactive intestinal peptide (VIP) is the avian prolactin releasing factor and changes in the concentrations of plasma prolactin (PRL) are found during the avian reproductive cycle. This study investigated the changes in the VIP/PRL system of native Thai hens rearing their young as compared to hens deprived of rearing their chicks. The number of VIP-immunoreactive (VIP-ir) neurons in the *Nucleus inferioris hypothalami* (IH) and *Nucleus infundibuli hypothalami* (IN) of hens rearing chicks (R) were compared with those of non-rearing chicks (NR). Plasma PRL levels were determined by enzyme-linked immunosorbent assay. The localization and number of VIP-ir neurons were determined by immunohistochemistry. The numbers of VIP-ir neurons in the IH–IN areas were high in the R hens, whereas the number of VIP-ir neurons decreased in the NR hens as compared to their respective R hens. During the rearing period, changes in the VIP-ir neurons within the IH–IN were correlated with plasma PRL levels. The results of the present study indicate for the first time that the VIP/PRL system plays a role in neuroendocrine reorganization to establish maternal behavior in native Thai chickens. The VIP/PRL system functions not only as a well established key regulator of incubation behavior, but is also involved in the regulation of rearing behavior. It is possible that VIP and the decline in the number of VIP-ir neurons and in turn VIPergic activity and the decrease in PRL levels are related to their contribution to rearing behavior of this non-seasonal breeding, equatorial precocial species.

Introduction

Two neuroendocrine systems play pivotal roles in the reproductive cycle in birds. One system involves chicken gonadotropin releasing hormone-I (cGnRH-I or GnRH) and the subsequent secretion of follicle stimulating hormone (FSH) and luteinizing hormone (LH; GnRH/FSH–LH system). A further system involves vasoactive intestinal peptide (VIP) and the subsequent secretion of prolactin (PRL; VIP/PRL system) with both systems influenced by dopamine (DA) (Bhatt et al., 2003; Chaiseha et al., 2003).

Changes in circulating PRL levels during the avian reproductive cycle have been well documented (El Halawani et al., 1988). Plasma PRL levels are low in reproductively quiescent birds, whereas levels increase in reproductively active laying hens. Circulating PRL levels are elevated throughout incubation (El Halawani et al., 1984; Sharp et al., 1989; Kosonsiriluk et al., 2008; Sartsoongnoen et al., 2008). In birds, it is very well established that PRL secretion is tonically stimulated (Kragt and Meites, 1965; Bern and Nicoll, 1968; Ben-Jonathan et al., 1989) and that VIP is the avian PRL releasing factor (PRF) secreted from neurons located in the infundibular nuclear complex (INF) of the caudo-medial hypothalamus (Sharp et al., 1989; El Halawani et al., 1997; Chaiseha et al., 1998; Chaiseha and El Halawani, 1999, 2005).

Abbreviations: AM, *Nucleus anterior medialis hypothalami*; DA, dopamine; ELISA, enzyme-linked immunosorbent assay; FSH, follicle stimulating hormone; GnRH, gonadotropin releasing hormone; HD, day of hatch; IH, *Nucleus inferioris hypothalami*; IHC, immunohistochemistry; IN, *Nucleus infundibuli hypothalami*; INF, infundibular nuclear complex; LH, luteinizing hormone; LHy, *Regio lateralis hypothalami*; ME, *Eminentia mediana* (median eminence); ML, *Nucleus mamillaris lateralis*; NR, non-rearing hens; nCPa, *Nucleus commissurae pallii*; nI, *Nucleus intramedialis*; PBS, phosphate buffered saline; PRF, prolactin releasing factor; PRL, prolactin; PVO, *Organum paraventriculare*; R, rearing hens; TH, tyrosine hydroxylase; V III, *Ventriculus tertius* (third ventricle); VIP, vasoactive intestinal peptide; VMN, *Nucleus ventromedialis hypothalami*.

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VIP, an octacosapeptide, was first isolated from the porcine duodenum (Said and Mutt, 1970). Subsequently, it has been found to be widely distributed in the central and peripheral nervous systems (Larsson et al., 1976; Rosselin et al., 1982), with high concentrations in the hypothalamus (Emson et al., 1978; Ceccatelli et al., 1991) and is considered to function as a neurotransmitter and neuroendocrine substance. In birds, VIP acts directly on the anterior pituitary gland (adenohypophysis) to stimulate PRL secretion during the reproductive cycle (Lea and Vowles, 1986; Macnamee et al., 1986; Proudman and Opel, 1988; El Halawani et al., 1990, 1997; Kosonsiriluk et al., 2008). In turkeys, the relationship between VIP contents in the median eminence (*Eminentia mediana*; ME), VIP mRNA steady-state levels within the hypothalamus, VIP levels in hypophyseal portal blood, and changes in circulating PRL levels, are correlated with the reproductive stages (Youngren et al., 1996; Chaiseha et al., 1998; Chaiseha and El Halawani, 1999). Immunocytochemical studies have shown that hypothalamic VIP-immunoreactive (VIP-ir) neurons within the INF and VIP-ir fibers in the ME correspond to enhanced circulating PRL levels in turkeys and native Thai chickens (Mauro et al., 1989; Kosonsiriluk et al., 2008). Other studies have also shown increased number and size of VIP-ir neurons within this region in domesticated pigeons and ring doves during periods of elevated circulating PRL levels (Péczely and Kiss, 1988; Cloues et al., 1990).

The results of immunohistochemical studies in the native Thai chicken, an equatorial species, have shown that VIP-ir neurons and fibers are extensively distributed throughout the brain. Changes in the number of VIP-ir neurons within the *Nucleus inferioris hypothalami* (IH) and *Nucleus infundibuli hypothalami* (IN) across the reproductive stages are observed and directly correlated with circulating PRL levels. These findings suggest that hypothalamic VIP expression in the IH–IN plays a regulatory role in year-round reproductive activity and indicates its importance in the regulation of reproductive activity in this equatorial species (Kosonsiriluk et al., 2008).

It has also been demonstrated that changes in the number of VIP-ir neurons in the IH–IN are associated with alterations in DAergic neurons within the *Nucleus intramedialis* (nI) and *Nucleus mamillaris* (ML), the release of PRL and the induction and maintenance of incubation behavior in native Thai chickens. It has been proposed that nesting activity stimulates PRL secretion by the activation of the DAergic system, which, in turn, stimulates VIP. The elevated PRL levels increase nesting activity and maintain incubation behavior (Prakobsaeng et al., 2011).

It is well known that PRL regulates maternal behavior in various species. The role of PRL in the induction and maintenance of maternal care has been extensively studied (Harris et al., 2004). In mammals, PRL begins to increase toward the end of gestation, when it is crucial for inducing milk production. In combination with progesterone and estrogen, PRL reduces the latency of onset of maternal behavior (Bridges and Ronsheim, 1990). In several birds: bantams (Sharp et al., 1979, 1988), mallard ducks (Goldsmith and Williams, 1980), domestic ducks (Hall and Goldsmith, 1983), Japanese bantams (Zadworny et al., 1988) and native Thai chicken (Kosonsiriluk et al., 2008) it has been shown that PRL concentrations are low before egg-laying, increase slightly during egg-laying, increase sharply before incubation and are maintained at high levels during incubation, and then decrease rapidly to basal levels immediately after hatching the young. Abundant evidence has linked maternal behavior in several avian species with increased PRL secretion. High PRL levels are known to be associated with brooding behavior in chickens (Sharp et al., 1979, 1988; Bedrak et al., 1981; Lea et al., 1981; Hoshino and Wakita, 1989), turkeys (Burke and Dennison, 1980; Proudman and Opel, 1981), mallard ducks (Goldsmith and Williams, 1980; Boos et al., 2007), and swans (Goldsmith, 1982). The studies in which broody chickens (Sharp et al., 1979), ducks (Goldsmith and Williams, 1980), and swans (Goldsmith, 1982) have been allowed to hatch and rear the young have shown that PRL concentrations decline at the end of the incubation period. Tactile stimuli from poults decrease circulating PRL in incubating hens without eggs and nests (Opel and Proudman, 1988). Physical contact, as well as visual and/or auditory stimuli from young chicks, is clearly involved in the appearance and maintenance of maternal behavior (Richard-Yris and Leboucher, 1987; Opel and Proudman, 1989). Furthermore, PRL has been implicated as a causative factor for reduced circulating gonadotropins and ovarian regression when birds shift from egg-laying to incubation behavior in chickens, turkeys, pigeons, pheasants, mallard ducks, cow birds, and native Thai chickens (Breitenbach and Meyer, 1959; Goldsmith and Williams, 1980; Bluhm et al., 1983; Lea and Sharp, 1989; Zadworny et al., 1989; El Halawani et al., 1997; Kosonsiriluk et al., 2008; Sartsoongnoen et al., 2008). It is apparent that PRL is involved in many aspects of reproductive physiology and behavior. It plays a pivotal role in parental behavior by mediating increases in incubation, crop milk production and secretion, feeding of young, and nest defense (Silver, 1984; Janik and Buntin, 1985; Lea et al., 1986; Buntin et al., 1991).

To date, the presence of hypothalamic VIP-ir, tyrosine hydroxylase-immunoreactive (TH-ir), and GnRH-I-ir neurons at different reproductive stage and throughout the incubation period have been reported in native Thai chickens (Kosonsiriluk et al., 2008; Sartsoongnoen et al., 2008, 2012; Prakobsaeng et al., 2011). However, no data are available that describe the interrelationship and the functional aspect of the changes in the VIPergic system with PRL levels during rearing behavior in native Thai chickens. The aim of this study was to investigate the correlation between plasma PRL levels and the VIPergic system in rearing (R) and non-rearing (NR) native Thai chickens. Comparisons were made in the number of VIPergic neurons in the IH and IN areas of R and NR birds. The findings of differential expression of VIP-ir neurons in IH–IN and their associated PRL levels may provide an insight into the role of the VIP/PRL system in the neuroendocrine regulation of rearing behavior.

Materials and methods

Experimental animals

Female native Thai chickens (*Gallus domesticus*), aged 18–20 weeks old, were used in the study. They were reared and housed with mature roosters (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 described in this study were approved by the Suranaree University of Technology Animal Care and Use Committee.

Experimental procedures

Experiment 1: effects of rearing behavior on plasma PRL levels

Blood samples were collected from the brachial vein of hens rearing chicks on the day of hatching, and weekly ($n = 6$) for 8 weeks after the day the chicks were hatched. Native Thai hens were divided into two groups to compare plasma PRL levels between hens rearing chicks (R) and hens removed from their chicks (NR). In the first group, hens were allowed to rear the chicks naturally. In the second group, chicks were removed from the hens immediately after the chicks were hatched. Blood samples ($n = 6$) were collected from the brachial vein of R hens and NR hens on the days the chicks were hatched, and weeks 1, 2, 3, 4, and 5 after hatching.