The Journal of Poultry Science, 42 : 87–100, 2005

≪**Review**≫

Neuroendocrinology of the Female Turkey Reproductive Cycle

Yupaporn Chaiseha[1] and Mohamed E. El Halawani[2]*

[1] School of Biology, Institute of Science, Suranaree University of Technology, Nakhon Ratchasima, Thailand

[2] Department of Animal Science, University of Minnesota, St. Paul, Minnesota, USA

A wealth of functional data confirms the involvement of hypothalamic vasoactive intestinal peptide (VIP) and gonadotropin releasing hormone (GnRH) in the regulation of the avian reproductive cycle. However, very little is known about the neurotransmitters or the anatomical locations of the hypophysiotropic neurons mediating the transition from one reproductive state to the next. Dopamine (DA) stimulates prolactin (PRL) and luteinizing hormone (LH) secretion by acting on VIP and GnRH neurons, respectively. DA may inhibit PRL secretion by antagonizing the action of VIP at the level of the pituitary, and limits LH secretion through presynaptic inhibition of GnRH release at the median eminence (ME) level. The stimulatory and inhibitory effects of DA are mediated via D_1 and D_2 DA receptors, respectively. However, the dopaminergic neuronal groups/subgroups which regulate the VIP/PRL and GnRH/LH systems remain to be clarified. Studies utilizing electro-pharmacological techniques in combination with radioimmunoassay, immunocytochemistry, and *in situ* hybridization histochemistry yield results suggesting the presence of a stimulatory dopaminergic pathway from the preoptic area (POA) to the infundibular nuclear complex (INF) area, where VIP neurons preside over the regulation of PRL secretion, as well as DA projections within the preoptic area-anterior hypothalamus (POA-AM) areas where the GnRH neurons reside that control LH secretion. DA neurons projecting to the ME which mediate the inhibition of the VIP/PRL and GnRH/LH systems remain to be identified.

Key words : birds, dopamine, gonadotropin releasing hormone, prolacin, vasoactive intestinal peptide

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Introduction

Two neuroendocrine systems play a pivotal role in the reproductive cycle of temperate zone birds, including the domestic turkey, which is used as a model in our laboratory. One neuroendocrine system involves chicken gonadotrophin releasing hormone-I (cGnRH-I, referred to as GnRH) and the subsequent secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH ; GnRH/LH-FSH) system and the other involves the prolactin (PRL) releasing factor (PRF), vasoactive intestinal peptide (VIP) and the subsequent secretion of PRL (VIP/PRL) system. The two systems are dependent upon the duration of daylight and are involved in the transduc-

Received : August 10, 2004, Accepted : September 4, 2004

* Corresponding author : Dr. Mohamed El Halawani, Department of Animal Science 495 AnSc/VetMed Building, 1988 Fitch Avenue, University of Minnesota St. Paul, MN 55108, USA

Tel : +1-612-624-9744 Fax : +1-612-625-2743 E-mail : elhal001@unm.edu

tion of photoperiodic information resulting in either gonad recrudescence and its associated sexual activity (egg laying), or gonad regression and termination of reproductive activity (photorefractoriness).

LH and FSH secretion and gene expression are stimulated by long day length (Nicholls *et al.,* 1988 ; Dawson *et al.,* 2001) and require the functional integrity of the GnRH neuronal system (Katz *et al.,* 1990 ; Sharp *et al.,* 1990). The increase in VIP/PRL secretion in response to long day length is gradual, but progressive, and both release and gene expression are up-regulated (Wong *et al.,* 1991 ; El Halawani *et al.,* 1996 ; Tong *et al.,* 1997). Activation of the GnRH/LH-FSH and VIP/PRL systems in the somatically mature photosensitive female turkey initiates the transition from reproductive quiescence to reproductive activity. Gonadotropins stimulate estrogen secretion (Wineland and Wentworth, 1975), inducing sexual receptivity (El Halawani *et al.,* 1986), and prime the VIP/PRL system to enhance PRL secretion (El Halawani *et al.,* 1983).

At the onset of sexual maturity (first ovulation), the preovulatory surge of progesterone induces the nesting behavior associated with oviposition (Wood-Gush and Gilbert, 1973 ; El Halawani *et al.,* 1986), and the combined action of estrogen, progesterone, and nesting activity further stimulates PRL secretion (El Halawani *et al.,* 1983, 1986). These increasing PRL levels suppress the activity of the GnRH/LH-FSH system (Rozenboim *et al.,* 1993 b ; You *et al.,* 1995), reducing ovarian steroids secretion (Porter *et al.,* 1991 ; Tabibzadeh *et al.,* 1995), terminating egg laying, inducing ovarian regression (Youngren *et al.,* 1991), and signal the transition from sexual behavior to incubation behavior. Elevated PRL levels and incubation behavior are maintained by tactile stimuli from the nest and eggs (El Halawani *et al.,* 1980, 1986 ; Opel and Proudman, 1989).

After hatching, or when eggs are replaced with poults, tactile stimuli from the young induces the emergence and maintenance of maternal responses, including the change from incubating eggs to brooding the young, vocalizations, nest desertion, a sharp decrease in circulating PRL (Opel and Proudman, 1989), molt, and the transition to the photorefractory state. With the onset of photorefractoriness, circulating PRL and LH levels and pituitary PRL/LH peptide and mRNA levels sharply decline, even though long day length continues (Wong *et al.,* 1991 ; Mauro *et al.,* 1992 ; Wong *et al.,* 1992 ; El Halawani *et al.,* 1996 ; Fig. 1). A rapid decrease in PRL and LH/FSH release and expression may be triggered at any time due to a lack of response to long day length (i.e. photorefractoriness) or by subjecting birds to short day lighting (Nicholls *et al.,* 1988 ; El Halawani *et al.,* 1990 a).

Immunoneutralization of VIP averts the rise in circulating PRL that follows photostimulation, prevents the induction of incubation behavior, up-regulates LHβ- and FSHβ subunit mRNAs, and extends the duration of reproductive activity (egg laying period), but does not prevent spontaneous gonad regression and molting (Sharp *et al.,* 1989 ; El Halawani *et al.,* 1995 a, 1996 ; Dawson and Sharp, 1998 ; Ahn *et al.,* 2001). Despite the well established antigonadotropic effect of PRL, it appears that the high circulating PRL level of laying, non-incubating birds is not the primary cause of

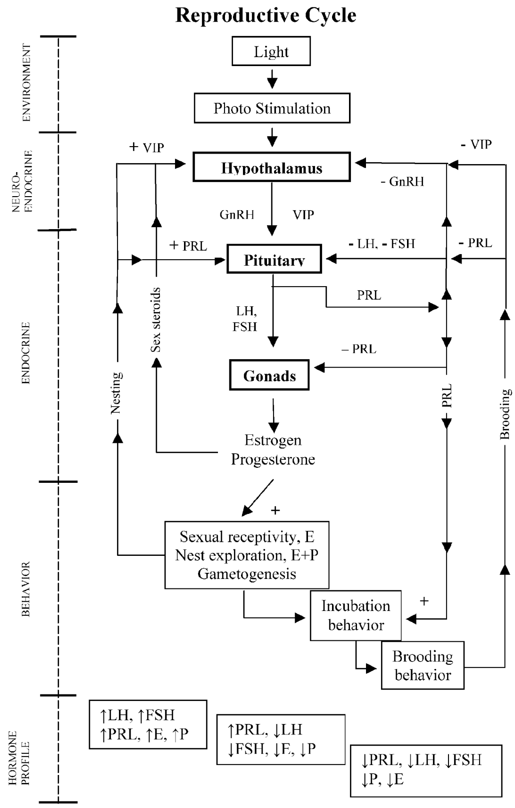

Fig. 1. Schematic diagram illustrating the interrelationships between external and internal stimuli and the neuroendocrine control of the reproductive cycle in the female turkey (bird).
+ =stimulation/positive feedback ; − =inhibition/negative feedback ; ↑ =increase ; ↓ =decrease ; GnRH=chicken gonadotropin releasing hormone-I ; VIP=vasoactive intestinal peptide ; LH=luteinizing hormone ; FSH = follicle stimulating hormone ; PRL = prolactin ; P = progesterone ; E=estrogen.

GnRH/gonadotropin suppression and the termination of reproduction (Juss,1993 ; Dawson and Sharp, 1998).

Neurohypophysiotropic Mechanisms

The final common pathway controlling the secretion of PRL, LH, and FSH is formed by a system of peptidergic neurons whose axons terminate around portal capillaries in the external layer of the median eminence (ME). VIP and GnRH are among the best characterized hypophysiotropic peptides.

GnRH neurons are found within the preoptic area (POA), anterior hypothalamus (AM) and lateral septum (LS ; Mikami *et al.,* 1988 ; Millam *et al.,* 1993). Little is known regarding the GnRH cell group(s) that project to the ME (Dawson and Goldsmith, 1997 ; Teruyama and Beck, 2000). Measurements of hypothalamic GnRH peptide in the hypothalamus during the reproductive cycle of the turkey (Millam *et al.,* 1989 ; El Halawani *et al.,* 1993 b ; Rozenboim *et al.,* 1993 a) indicate that levels do not change in incubating birds. The amount of GnRH in the hypothalamus decreases during photorefractoriness in the turkey (Rozenboim *et al.,* 1993 a) and other avian species (Dawson *et al.,* 1985 ; Foster *et al.,* 1987 ; Bluhm *et al.,* 1991 ; Saldanha *et al.,* 1994 ; Hahn and Ball, 1995). In a recent study from our laboratory (Kang *et al.,* 2004), GnRH mRNA expression was determined utilizing *in situ* hybridization histochemistry (ISH) during the four different reproductive stages of the female turkey. GnRH mRNA was highly expressed in the organum vasculosum laminae terminalis (OVLT) and the bed nucleus of the pallial commissure (nCPA), and limited expression was observed in the POA, medial preoptic nucleus (POM), and LS. Hypothalamic GnRH mRNA expression was significantly increased after subjecting the non-photostimulated female turkey to a 90 minute light period at Zeitgeber time (ZT) 14. GnRH mRNA abundance within LS, OVLT, and nCPA areas was highest in laying hens, with decreasing abundance found in non-photostimulated and incubating hens, respectively. The lowest levels of GnRH mRNA were observed in photorefractory hens. These results indicate that hypothalamic GnRH mRNA expression may be used to precisely characterize the different reproductive states.

VIP neurons are widely distributed throughout the hypothalamus (Yamada *et al.,* 1982 ; Mikami and Yamada, 1984 ; Peczely and Kiss, 1988 ; Mauro *et al.,* 1989 ; Chaiseha and El Halawani, 1999). Studies using a combination of electrophysiology, radioimmunoassay, immunocytochemistry (ICC), and ISH suggest that VIP in the ME is derived from neurons located within the infundibular nuclear complex (INF ; Macnamee *et al.,* 1986 ; Mauro *et al.,* 1989 ; Chaiseha and El Halawani, 1999 ; Youngren *et al.,* 2002 a). VIP is very well accepted as the avian PRL releasing factor (PRF ; El Halawani *et al.,* 1997). VIP peptide and mRNA levels in the INF increase following exposure to long days and remain elevated as long as such exposure continues, declining only when the bird is subjected to short days (Mauro *et al.,* 1989 ; El Halawani *et al.,* 1997 ; Chaiseha and El Halawani, 1999). ICC and ISH studies have shown that fluctuations in hypothalamic VIP immunoreactivity and expression within the INF parallel fluctuations in circulating PRL (Mauro *et al.,* 1989 ; Chaiseha and El Halawani, 1999). Other studies have also shown increases in the number and size of VIP immunoreactive neurons within this region in the domesticated pigeon following the initiation of crop milk secretion and feeding of off-spring, which are periods of elevated circulating PRL (Peczely and Kiss, 1988). Moreover, concentrations of VIP in portal blood plasma are significantly higher than VIP concentrations in peripheral blood plasma in all reproductive stages. VIP concentrations in portal blood plasma are lowest in non-photostimulated, reproductively quiescent turkey hens, and highest in incubating hens, with laying and photorefractory hens having intermediate levels (Youngren *et al.,* 1996 a). These differences in VIP portal blood concentrations mirror those of PRL in the general circulation, supporting the hypothesis that VIP is the avian PRF.

A decoupling of total hypothalamic VIP peptide and mRNA from circulating and pituitary PRL is seen in the VIP/PRL system of reproductively inactive photo-refractory birds (Mauro *et al.,* 1992 ; Saldanha *et al.,* 1994 ; Chaiseha *et al.,* 1998 ;

Chaiseha and El Halawani, 1999). PRL reaches its lowest level and VIP its highest level during the photorefractory stage of the reproductive cycle. This raises several questions related to the role of VIP in the initiation and termination of the avian reproductive cycle. Does the elevated hypothalamic VIP expression indicate an enhanced VIPergic system ? If so, which VIP neuron groups are involved ? VIPergic neuron ensembles are found in the INF, POA, lateral septal organ (LSO), and anterior hypothalamus-suprachiasmatic nucleus area (AM-SCN ; Mauro *et al.,* 1989 ; Chaiseha and El Halawani, 1999). We have established that VIP neurons residing in the INF area are the source of VIP regulating PRL secretion (Mauro *et al.,* 1989 ; Chaiseha and El Halawani, 1999 ; Youngren *et al.,* 2002 a). VIP axon terminals have been found in close apposition to GnRH neurons in the LSO and POA (Teruyama and Beck, 2001), and an inverse relationship between VIP in the INF and GnRH in the POA has been reported (Deviche *et al.,* 2000). Elevated hypothalamic VIP peptide and mRNA contents are associated with gonad regression and suppression of gonadotropin in photorefractory turkeys (Mauro *et al.,* 1989 ; Chaiseha *et al.,* 1998 ; Chaiseha and El Halawani, 1999). VIP immunoneutralization up-regulates LHβ- and FSHβ subunit mRNAs (Ahn *et al.,* 2001), and delays the onset of photorefractoriness and molt in starlings (Dawson *et al.,* 1998). While the functional significance of these findings remains to be clarified, they imply that VIP exerts an inhibitory influence on the gonadotropin system. There are indications that VIP has a central inhibitory influence on GnRH/LH release (Pitts *et al.,* 1994).

Monoaminergic Regulation

A wealth of functional data has confirmed the involvement of hypothalamic VIP and GnRH in the regulation of the avian reproductive cycle (El Halawani *et al.,* 1990 a, 1990 b ; Sharp *et al.,* 1998). However, very little is known about the neurotransmitter system(s) and the anatomical location of the hypophysiotropic group/sub-group of neurons regulating GnRH/LH-FSH and VIP/PRL systems and mediating the transition from one reproductive state to the next. This review on the monoaminergic regulation of GnRH/LH, FSH and VIP/PRL systems is limited to the recent advances in dopaminergic and serotonergic mechanisms. Earlier work on the subject has been reviewed elsewhere (El Halawani *et al.,* 1984, 1988 ; El Halawani and Rozenboim, 1993 a)

Dopamine Neurotransmission

Several DA cell groups have been identified in the preoptic-hypothalamic areas (Kiss and Peczely, 1987 ; Reiner *et al.,* 1994). DA has a stimulating effect on PRL and LH secretion by acting on their respective neuropeptidergic neurons (Bhatt *et al.,* 2002). The DAergic system has also been shown to inhibit the stimulatory effect of VIP on PRL secretion at the pituitary level (Youngren *et al.,* 1998 a), apparently by releasing DA into the capillaries of the hypophysial portal system (Youngren *et al.,* 1996 a). DAergic neurons inhibit GnRH release through presynaptic inputs at the ME level, as has been demonstrated in the chicken (Contijoch *et al.,* 1992 ; Fraley and Kuenzel, 1993).

Dopaminergic neurons are not located in a single discrete hypothalamic nucleus or region, but instead are dispersed among a variety of hypothalamic regions (POA-AM), suprachiasmatic nucleus (SCN), lateral hypothalamic area (LHy), paraventricular nucleus (PVN), lateral mamillaris nucleus (ML), and dorsomedial nucleus (DM ; Kiss and Peczely, 1987 ; Reiner *et al.,* 1994). Given their widespread distributions, and the findings that DA axons and terminals are found intermingled with VIP neurons in the INF, GnRH neurons in the POA, and with both VIP and GnRH terminals in the external layer of the ME (preliminary data), it is reasonable to consider whether any regional specificity exists in those DA neurons that is neuroendocrine in nature, i.e., controlling the release and expression of VIP/PRL and GnRH/LH-FSH.

An advance in elucidating the neurochemical mechanisms came from our laboratory where using the turkey as a model, we were able to demonstrate the dual role of DA in PRL secretion and expression, stimulating via D_1 DA receptors and inhibiting via D_2 DA receptors (Youngren *et al.,* 1995 ; Xu *et al.,* 1996 ; Chaiseha *et al.,* 1997 ; Youngren *et al.,* 1998 a). Both D_1 and D_2 DA receptor mRNAs are abundant in the brain and pituitary (Schnell *et al.,* 1999 a), suggesting DA exhibits biphasic actions within the turkey hypothalamus and pituitary. In fact, tonic stimulation of PRL secretion and gene expression are regulated centrally via D_1 DA receptors on VIP neurons, where the expression of D_1 DA receptors is greater (6-fold) than that of D_2 DA receptors (Youngren *et al.,* 1995 ; Chaiseha *et al.,* 2003). DA inhibits PRL secretion and gene expression by blocking the action of VIP at the level of the anterior pituitary via D_2 DA receptors (Youngren *et al.,* 1998 a).

The increase in circulating PRL levels in response to long days is a gradual and incremental process associated with gonad recrudescence and egg laying and culminating in the dramatic augmentation observed at the onset of incubation (Goldsmith, 1985 ; El Halawani *et al.,* 1990 a). This slow progressive photostimulated increase in PRL level stands in stark contrast to the sharp and immediate decline in circulating PRL that occurs in an incubating hen following hatching of its eggs or an occurrence of nest deprivation (El Halawani *et al.,* 1980 ; Opel and Proudman, 1989). This precipitous drop in circulating PRL is most likely related to the activation of an inhibitory neural system that overrides the tonic stimulation of PRL by VIP. The cause of this inhibition is surmised to be DA, acting at the pituitary level to block the tonic stimulation of VIP upon lactotrophs (Youngren *et al.,* 1998 a), since D_2 DA receptor mRNA expression by anterior pituitary lactotrophs is up-regulated at this stage of the reproductive cycle (Chaiseha *et al.,* 2003). The identification of DAergic neurons which project to the ME and deliver DA to the anterior pituitary and those which project to the INF and stimulate VIP is the subject of ongoing studies. It remains undetermined whether the physiological processes involved in the termination of reproductive activity and the following insensitivity to long day lengths in non-incubating birds are connected to the same neural mechanisms that are responsible for the sharp decline in PRL during the transition from incubation to photorefractoriness. In the turkey, as in single brooded species in temperate zones, gonadotropins secretion may not increase after the young hatch because of the development of photorefractoriness (Follett, 1984 ; Wingfield and Farner, 1993).

The marked decrease in PRL release and gene expression and the insensitivity to long day lighting that is characteristic of photorefractory birds is apparently not attributable to the VIP/PRL system. Microinjection of a D_1 DA receptor agonist into the INF area of the hypothalamus increases plasma PRL levels equally in both laying and photorefractory hens (Youngren *et al.,* 2002 a), suggesting that the cells that secrete VIP and PRL are fully responsive at the time when photorefractoriness becomes apparent. Their inactivity presumably reflects either the inability of hypothalamic DAergic neurons (Kiss and Peczely, 1987 ; Reiner *et al.,* 1994) projecting to the INF (Youngren *et al.,* 2002 a) to stimulate the VIP/PRL system and/or the switching on of inhibitory DAergic neurons that initiate the shutting down of PRL secretion by activating D_2 DA receptors at the pituitary level (Schnell *et al.,* 1999 a ; Schnell *et al.,* 1999 b). This is substantiated by the findings that : 1) activation of D_2 DA receptors in the anterior pituitary inhibit the stimulatory effect on PRL secretion of VIP infusion into the anterior pituitary or of electrical stimulation (ES) in the POA (Youngren *et al.,* 1996 b ; Youngren *et al.,* 1998 a ; El Halawani *et al.,* 2000) ; 2) the sharp and immediate decline in PRL secretion after the young hatch or following nest deprivation occurs despite the presence of high pituitary PRL levels, which can be released at an enhanced rate by VIP *in vitro* or by *in vivo* electrical stimulation in the POA (El Halawani *et al.,* 1990 b ; Youngren *et al.,* 1993) ; and 3) the up-regulation of D_2 DA receptor mRNA in the anterior pituitary of hypoprolactinemic photorefractory hens (Chaiseha *et al.,* 2003). It can be argued that the inhibition of VIP release, and in turn PRL secretion, may be the result of down- or up-regulation of D_1 and D_2 DA receptors, respectively, on VIP neurons located in the INF area (Mauro *et al.,* 1989 ; Chaiseha *et al.,* 1997 ; Chaiseha *et al.,* 2003).

The role of DA in gonadotropic regulation remains controversial since both stimulatory and inhibitory effects have been reported (El Halawani *et al.,* 1988 ; Sharp *et al.,* 1998). Using the measurement of circulating levels of LH as the end point may in part have contributed to these inconstant results. Circulating LH levels are low and the LH response to physiological manipulation is small and variable (Youngren *et al.,* 1993), as compared to PRL, for example, making interpretation difficult. We are now able to measure both LHβ- and FSHβ subunit mRNA contents, which display a robust and stable response to physiological manipulation (Ahn *et al.,* 2001 ; Bhatt *et al.,* 2003). Recent data from our laboratory indicate that DA stimulates LHβ subunit mRNA (Bhatt *et al.,* 2003), whereas earlier data indicated that DA inhibited GnRH release at the ME level (Contijoch *et al.,* 1992 ; Fraley and Kuenzel, 1993). The possibility remains that DA may have both stimulatory and inhibitory influences on the GnRH/gonadotropic system, depending upon the site of action and/or the DAergic receptor subtypes involved, as is the case with VIP/PRL.

Serotonin Neurotransmission

Considerable evidence indicates that the serotonergic (5-HTergic) system is a potent stimulator of PRL secretion in birds (Hall *et al.,* 1986 ; El Halawani *et al.,* 1988). 5-HT seems to act centrally since ; 1) it has no effect on PRL secretion when added to pituitary cells *in vitro* (El Halawani *et al.,* 1988) ; 2) 5-HT receptors are not present in the anterior pituitary (Macnamee and Sharp, 1989) ; and 3) intraventricular infusion of 5-HT causes plasma PRL to increase in turkeys (EI Halawani *et al.,* 1995 b ; Pitts *et al.,* 1996). We (Youngren *et al.,* 1989) have suggested that 5-HTergic fibers, traversing the hypothalamic VMN, stimulate PRL secretion through interneuronal DAergic connections to the INF where the majority of VIP immunoreactive neurons are found (Mauro *et al.,* 1989 ; Chaiseha and El Halawani, 1999). 5-HT neurons are part of a common pathway, presumable residing within the avian hypothalamus, which stimulates the secretion of PRL from the anterior pituitary. When D_1 DA receptors are blocked, the PRL-releasing efficacy of not only DA (Youngren *et al.,* 1998 a), but also 5-HT (Youngren *et al.,* 1998 b), is suppressed. 5-HT receptors appear to lie above the synapse containing D_1 DA receptors. As indicated above, the primary PRF (perhaps the only one) released from the hypothalamus into the hypothalamo-pituitary portal vessels is VIP (El Halawani *et al.,* 1997). The ability of 5-HT and DA to stimulate PRL secretion is contingent upon an intact VIPergic system. When birds are immunized against their own VIP, the central infusion of 5-HT (EI Halawani *et al.,* 1995 b), or DA (Youngren *et al.,* 1996 b) can not stimulate PRL secretion. The infusion of VIP into the ME of immunized birds results in a severely curtailed PRL response. Thus, 5-HT, DA, and VIP act to stimulate PRL secretion via a common pathway expressing 5-HTergic, DAergic, and VIPergic receptors at synapses arranged successively in that functional order.

In a preliminary study (Youngren and El Halawani, 2002 b), bilateral microinjections of 5-HT in the caudal VMN of the hypothalamus, but not the rostral part, notably impeded the PRL release effected by electrical stimulation in the POA. These data lead us to the hypothesis that 5-HT, at least at the VMN level, may be involved in the decline in circulating PRL observed during reproductive inactivity i.e. the photorefractory state (Youngren and El Halawani, 2002 b). Also, recent data from our laboratory (El Halawani *et al.,* 2004), show that electrical stimulation in the POA, which is known to stimulate LH and PRL secretion, activates GnRH and VIP immunoreactive neurons (as indicated by c-fos mRNA expression) in the POA and INF areas, respectively. This was associated with an activation of a DAergic neuronal group residing in the ML area of the hypothalamus. This is the first identification of a specific DA group that is associated with the stimulation of GnRH/LH,FSH and VIP/PRL systems.

How the hypothalamic 5-HT-DA-VIP pathway may alter PRL secretion is unknown. A hypothetical mechanism is proposed below, (Fig. 2).

1) PRL secretion is tonically stimulated by VIP neurons in the INF (El Halawani *et al.,* 1997).
2) 5-HTergic fibers, traversing the hypothalamic VMN/ML, stimulate PRL secretion through interneuronal DAergic projections, probably from the VMN/ML to the INF (Mauro *et al.,* 1989 ; Chaiseha and El Halawani, 1999 ; Youngren *et al.,* 2002 a).
3) The tonic stimulation of PRL release and expression is inhibited by DA at the pituitary level via D_2 DA receptors (Youngren *et al.,* 1995 ; El Halawani *et al.,* 1997). The source of DA is unknown. DA neurons in the VMN/ ML

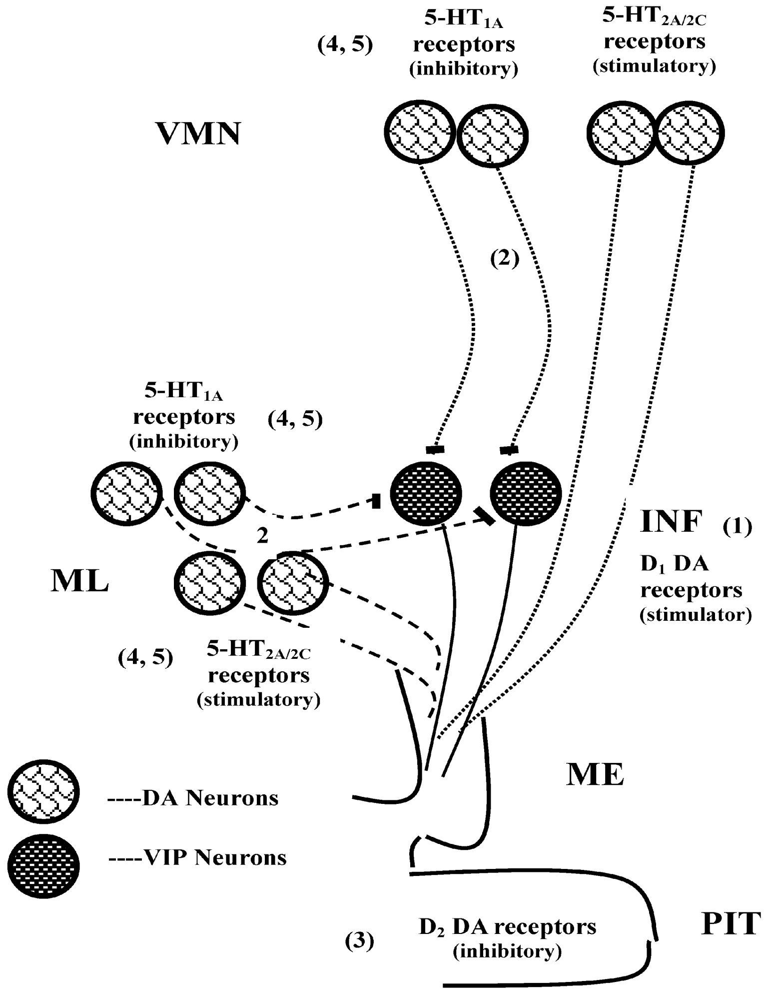

Fig. 2. Schematic diagram demonstrating the interrelationships between 5-HT, DA and the control of VIP/PRL secretion.

(Kiss and Peczely, 1987 ; Reiner *et al.,* 1994) may or may not be the source.

4) 5-HT modulation of the VIP/PRL system requires a functional DAergic system (Youngren *et al*., 1998 b).
5) 5-HT_{1A} and 5-$HT_{2A/2C}$ receptors mediate the inhibitory and stimulatory influences of 5-HT on PRL secretion, respectively (unpublished preliminary results).
6) The microinjection of 5-HT in the VMN inhibits PRL release induced by electrical stimulation in the POA (Youngren and El Halawani, 2002 b).

Accordingly, the gradual increase in PRL secretion associated with photostimulation and reproductive activity is related to tonic DA stimulation of VIP neurons in the INF via D_1 DA receptors. The source of DA appears to be DAergic neurons located in the ML.

The abrupt decline in circulating PRL levels associated with the start of ovarian regression and the ending of egg laying, as in photorefractory birds, reflect : 1) the inability of the DAergic neurons in the ML and/or VMN to stimulate the infundibular VIP system ; and/or 2) the activation of DAergic neurons projecting to the ME which turn off PRL secretion by activating D_2 DA receptors at the pituitary level. The modification in DAergic neurotransmission in the ML and/or VMN is mediated by inhibitory 5-HT_{1A} and stimulatory 5-$HT_{2A/2C}$ receptors on these DA cells.

Acknowledgement

Supported by The Thailand Research Fund (TRF) #RSA4780001 to (YC) and BARD #US-3399-03C and USDA #2004-35203-14771 to (MEH).

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