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Studies on the Endocrine and Neuroendocrine Control of Broodiness in the Yuehuang Hen

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Abstract: The hypothesis that serotonin (5-HT) and dopamine stimulate prolactin (PRL) release, either directly from the pituitary gland or by acting through Vasoactive Intestinal Peptide (VIP), was investigated by assessing the role of hypothalamic dopamine and 5-HT in the control of broodiness and PRL release in Cantonese native chicken breed called the Yuehuang hen. A second objective was to assess the involvement of hypothalamic VIP in the control of broodiness and PRL release by using dopamine and serotonin receptor antagonists respectively. In Experiment 1, two hundred laying hens from battery cages were transferred to floor pens with nest boxes to induce broodiness. Starting from the second day after the onset of broodiness, sixty hens were allotted into groups 1, 2 and 3 respectively, corresponding to chlorpromazine-treated (n = 20), cyproheptadine-treated (n = 20) and control (n = 20) groups. Blood samples were collected from the wing veins of hens in each group on days 2, 5, 9, 14 and 19 respectively after the onset of broodiness for radioimmunoassay of PRL and Luteinizing Hormone (LH). Results indicate that in the drug treated hens which terminated broodiness, the concentration of plasma PRL decreased significantly ($p<0.01$) while the concentration of plasma LH increased significantly ($p<0.05$) as compared to the control hens. The plasma PRL levels showed no significant ($p>0.05$) changes between the chlorpromazine and cyproheptadine treated hens. Within the chlorpromazine and cyproheptadine treated hens, there were significant ($p<0.05$) changes in plasma PRL levels between day 2 and days 5, 9, 14 and 19. The plasma PRL levels in the control hens showed no significant ($p>0.05$) changes throughout the blood sampling periods. Sixteen (80%) chlorpromazine treated hens terminated broodiness on an average of 4.6±0.8 days but four hens (20%) did not. However, thirteen (65%) cyproheptadine treated hens terminated broodiness on an average of 2.3±0.2 days while seven (35%) did not. In Experiment 2, sixty animals were used but their management and drug treatment for group 1 (n = 10) and group 2 (n = 10) were the same as described in Experiment 1. Group 3 (n = 10) served as the control. Eight hens each from groups 1, 2 and 3 were randomly selected for immunohistochemical studies. On day 7 after the onset of broodiness, hypothalamus from anaesthetized chlorpromazine and cyproheptadine treated hens as well as control hens were processed for immunohistochemical localization of VIP neurons in the hypothalamus of Yuehuang hens. Morphological observation showed a higher number of VIP neurons in the hypothalamus of the control hens. A few VIP neurons which were very faint were also found in the hypothalamus of the chlorpromazine and cyproheptadine treated hens. Results of these studies indicate a relationship between the functions of dopamine and 5-HT neurons in the hypothalamus and reproductive activities in domestic hens. They are consistent with the view that hypothalamic dopamine and 5-HT are regulators of PRL release and that using drugs which inhibit the functional activities of these neurotransmitters can inhibit PRL release to disrupt broodiness in hens to maintain egg production. The results also indicate a causal relationship between hypothalamic VIP and changes in PRL secretion associated with reproductive activities in domestic hens. This is consistent with the view that VIP might be an important hypothalamic PRL releasing neuropeptide and also indicate that VIP might be a physiological PRL regulatory hormone in domestic hens.

Key words: Broodiness, prolactin, vasoactive intestinal peptide, luteinizing hormone, dopamine, serotonin, chlorpromazine, cyproheptadine

INTRODUCTION

Broodiness describes the inherent behaviour displayed most frequently by domestic hens after they have laid a clutch of eggs which would be incubated under feral conditions. The development of broodiness is induced by interactions between the environment, genotype and

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the endocrine system (Sharp, 1989). Convincing evidence (El Halawani *et al*., 1991a,b; Youngren *et al*., 1991) have been presented implicating increased PRL secretion as a cause of reduced circulating gonadotropins, ovarian regression and the shift from egg laying to the broody phase of reproduction.

The main functions of the endocrine system that lead to the development of broodiness are an increase in the concentration of plasma PRL (Sharp *et al*., 1988; Etches and Cheng, 1982) and as egg laying stops, a fall in plasma LH and ovarian steroids. The increase in plasma PRL plays a role in the initiation and maintenance of broody behaviour (Janik and Buntin, 1985) and in the suppression of gonadotropin release (Lea *et al*., 1981).

The onset and maintenance of broody behaviour is dependent upon the nest entry induced progesterone surges in laying hens (El Halawani *et al*., 1991a,b). This indicates that nest entry and persistent nesting are prerequisite for the PRL induction of broody behaviour which triggers the elevated PRL levels which maintain broody activity. Tactile stimulation from the brood patch which is provided by the eggs or floor of the nest site stimulates PRL secretion since the removal of a broody hen from its nest results in rapid decrease in plasma PRL (Sharp *et al*., 1988). This observation indicates that once broodiness is initiated, it is maintained only by high levels of plasma PRL. This is supported by the fact that injection of ovine PRL to broody bantam hens deprived of their nests maintained readiness to incubate (Sharp *et al*., 1988). PRL secretion in birds is predominantly regulated by releasing factors of which the best characterized are VIP, 5-HT and dopamine (Hall *et al*., 1986).

The most important neuroendocrine influence on PRL secretion in birds may be attributed to VIP which is secreted from the hypothalamus. VIP is produced by neurons in the medial basal hypothalamus and external median eminence. It is then transported in the portal vascular system to the pituitary gland, where it stimulates the secretion of PRL from the lactotrophic cells (Etches, 1996). The regulatory mechanisms for increased PRL secretion in broody hens appear to involve modulation at the level of both the hypothalamus and pituitary. The medial basal hypothalamus contains the final neural elements that regulate anterior pituitary secretion of PRL in the turkey (El Halawani and Rozenboim, 1993).

Hypothalamic VIP increases PRL secretion from chickens and turkey pituitary glands particularly when the pituitary gland responsiveness is enhanced with estrogen pre-treatment (Sharp *et al*., 2005). The role of the anterior pituitary gland in the expression of changes in the circulating PRL and LH levels associated with the reproductive cycle of turkeys was investigated by El Halawani *et al*. (1988a). It was found that the anterior pituitary of turkeys appear to have a role in the induction of changes in circulating PRL associated with reproductive cycle. The low LH level found was attributed to minimal hypothalamic induction of LH release during the various stages of the reproductive cycle. The hypothesis that during incubation, female turkeys exhibit elevated circulating PRL which may be the result of enhanced pituitary responsiveness to VIP induces an increase in LH secretion in all reproductive groups (Sharp *et al*., 2005). This shows that VIP enhances Luteinizing Hormone Releasing Hormone (LHRH) induced LH secretion. This shows that PRL secretion *in vitro* by pituitary cells from turkeys in various reproductive stages reflected the circulating levels of PRL at these stages. PRL release in turkeys may be modulated by gonadal steroids acting directly on the cells of anterior pituitary (Knapp *et al*., 1988). Therefore, these steroids probably enhance the response of the lactotrophs to neuropetide induction of PRL release by increasing PRL synthesis and storage. Immuno-neutralization of VIP can inhibit PRL release so as to inhibit the development of broodiness and maintain egg laying in birds. Daily injection of cVIP antibody led to broody hens deserting their nests within 3-5 days (Sharp *et al*., 1989). This shows that the VIP antibodies in the peripheral circulation were able to immuno-neutralize VIP effectively in the hypophysial vasculature. Active immunization of turkeys against VIP (El Halawani *et al*., 1995) starting on the day of photo-stimulation with cVIP conjugated to keyhole limpet haemocyanin depressed PRL release compared to the controls. Chen *et al*. (1997) actively immunized Taihe hens with the conjugate of c-terminal fragment of VIP and Bovine Serum Albumen (BSA) to study the effect on their reproductive performance. The results indicate that active immunization against VIP decreased plasma PRL and broody rate, and consequently maintained egg production.

The neural mechanisms that govern increased PRL secretion in broody hens appear to involve monoaminergic systems and that both hypothalamic 5-HT and dopamine have been implicated (El Halawani and Rozenboim, 1993). Serotonin (5-HT) facilitates the release of pituitary PRL in turkey hens when they are treated with methysergide, a 5-HT receptor blocker, and prevents the increase in PRL in response to electrical stimulation of the hypothalamus (El Halawani *et al*., 1988b). This substantiates the proposition that 5-HT is a central neurotransmitter that stimulates PRL secretion (Hall *et al*., 1986). Serotonin is also involved in mediating a nesting stimulated increase in PRL secretion and maintenance of broody behaviour. PRL is secreted by the administration of serotonin or drugs which mimic its action in the brain (Macnamee and Sharp, 1989a). There is also an increase in the turnover of serotonin in the hypothalamus of broody turkey hens (El Halawani and Burke, 1976) while systemic or cerebro-ventricular injection of serotonin, serotonin precursor, or serotonin agonist, stimulate PRL release

(Hargis and Burke, 1984). The administration of serotonin synthesis inhibitor, p-chlorophenylalanine (PCPA) inhibits PRL release in re-nesting and broody turkeys (El Halawani *et al*., 1983).

The stimulatory role of dopamine in the regulation of PRL release was investigated by the administration of low doses of dopamine directly into the brain of broody turkey hens (Macnamee and Sharp, 1989b). This resulted in increased turnover of hypothalamic dopamine. The turnover of dopamine increased in the hypothalamus of turkeys during the transition from laying to incubation (El Halawani and Burke, 1976).

The present study was undertaken to: (1) investigate the role of hypothalamic dopamine and serotonin (5-HT) in the control of broodiness and PRL release in the Yuehuang hen and (2) assess the involvement of hypothalamic VIP in the control of broodiness and PRL release by using dopamine and 5-HT receptor antagonists.

MATERIALS AND METHODS

Animals, management and sampling

Experiment 1: Two hundred laying Yuehuang hens, fifty weeks old and about 2.0 kg live weight, from the South China Agricultural University Experimental Poultry Farm and housed in battery cages were randomly selected and transferred to floor pens (2.5 m x 2.0 m) containing nest boxes to induce broodiness. The birds were kept in groups of five. All eggs laid by the hens were removed daily. The hens were exposed to natural lighting conditions and they had free access to water and feed. Hens which expressed signs of broodiness were taken for the experiment. Broodiness was recognized as persistent nesting combined with characteristic clucking. Starting from the second day after the onset of broodiness, each hen in group 1 (n = 20) was orally treated with 150 mg chlorpromazine (dopamine receptor antagonist) per day until the termination of broodiness. Each hen in group 2 (n = 20) was orally treated with 20 mg cyproheptadine (5-HT receptor antagonist) per day until the termination of broodiness and hens in group 3 (n = 20) served as the control. However, drug treatment continued for the hens which did not terminate broodiness until day 7 and it was stopped.

Blood samples were collected from the wing veins of hens in groups 1 to 3 on days 2, 5, 9, 14 and 19 respectively after the onset of broodiness. The blood samples were centrifuged and stored at -20°C until assayed for PRL and LH.

Experiment 2: Sixty Yuehuang hens, fifty weeks old and about 2 kg live weight, from the South China Agricultural University Experimental Poultry Farm and housed in battery cages were randomly selected and used for the experiment but their management and drug treatment for group 1 (n = 10) and group 2 (n = 10) were the same as described in experiment 1. Group 3 served as the control (n = 10). On days 2 and 7 after the start of broodiness for the control group and on day 7 for the groups 1 and 2 hens which terminated broodiness within 2 to 5 days following drug treatment, 8 hens in each group were killed for immunohistochemical localization of VIP in the hypothalamus. The hens were anaesthetized by intra-muscular (i.m) injection of 0.5 mg ketamine and jugular arteries were exposed. Forty (40) ml 0.1M PBS (pH 7.3) was infused into the arteries followed by 80 ml phosphate buffered 4% formaldehyde (pH 7.3) which served as a fixative. Hypothalamus was dissected out and fixed in the above mentioned fixative for immunohistochemical detection of VIP neurons.

Hormone assays

Radioimmunoassay of LH: To 5 μl of USDA-cLH-1-3 (LH tracer) in plastic iodination vial was added 20 μl of 0.25 mol/L phosphate buffer to dissolve the content. This was followed by the addition of 0.5 mCi of high specific activity $Na^{123}I$. After that, 10 μl of 0.25 mol/L phosphate buffer containing chloramine T (58 mg/ml) was added to initiate the iodination reaction. The reaction was allowed to proceed for 5 min and finally ended by the addition of 100 μl of transfer solution (composed of 1% KI, 16% sucrose and 0.01% bromophenol blue). This was followed by the addition of 200 μl of 10% Bovine Serum Albumin (BSA) to the iodination vial. The iodination reaction mixture was then removed and loaded onto a Sephadex G-25 column, eluted with 0.05 mol/L phosphate buffer (pH 7.4). Eight-drop fractions were collected in eight test tubes until the dye was completely eluted from the column. The fraction containing the first peak of radioactivity was used as tracer for the radioimmunoassay. The assay buffer was 0.05 M Phosphate Buffered Saline (PBS) solution (pH 7.4) enriched with 0.1% BSA. Duplicate plasma samples and aliquots of standard (USDA-cLH-K-3) comprising 16, 8, 4, 2, 1, 0, 5, 0.25, 0.125, 0.061 and 0.03 ng/ml were dissolved in assay buffer with 2% (v/v) broody hens plasma. To each of the tubes was added 100 μl assay buffer plus another 400 μl PBS to make a volume of 0.5 ml. After that, 200 μl of 0.05M PBS containing 0.05M EDTA and 0.5% normal rabbit serum (first antibody) at a dilution of 1:12500 was added to each of the standard and the sample tubes except non-specific binding. This was followed by the addition of 100 μl of ^{125}I-LH (labelled LH) in assay buffer and the mixture incubated at 4°C for 48 h. After that, 100 μl of donkey anti-rabbit antiserum (second antibody, purchased from the Northern Institute of Immunology, Beijing) at 1:20 dilution in PBS was added to each tube except Total. The tubes were incubated at 4°C for 24 h. This was followed by the addition of 2.0 ml of spinning down buffer (0.05M PBS plus 6% polyethylene glycol of molecular weight 6000) to all tubes except Total and centrifuged at 1900 g for 30 min in a refrigerated centrifuge at 4°C. The supernatants were discarded and the radioactivity in the precipitates counted in a gamma counter to determine the LH concentration in each sample.

Radioimmunoassay of PRL: The method for radio-iodination and the procedure for radioimmunoassay were the same as in radioimmunoassay of LH above except that the reference standard and the concentrations of the first and second antibodies were different. The chicken PRL tracer used for radio-iodination was AFP-4444B. Duplicate serum samples and aliquots of standard (AFP-10328B) comprising 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78, 0.39 and 0.19 ng/tube was used. Two percent (2%) normal rabbit serum (AFP-151040789) in 0.1 mol/L PBS (pH 7.4) at 1:50000 dilution was used as the first antibody while donkey anti-rabbit antiserum at 1:4 dilution was used as the second antibody.

Immunohistochemical localization of VIP in the hypothalamus: Following fixation of the hypothalamus in phosphate buffered 4% formaldehyde (pH 7.3) for 24 h, the tissue blocks were dehydrated in solutions of alcohol of increasing concentration. Xylene was used as inter-medium before the tissues were embedded in hot paraffin. The tissues were sectioned transversely (15 µm) on a tissue slicing microtome and processed for immunohistochemical localization of VIP using Peroxidise-Antiperoxidase Procedure (PAP). The primary antibody, sheep anti-VIP (6DL31/4) was used at a dilution of 1:4000. Rabbit anti-goat antibody (2454, Dako) at a dilution of 1:150 was used as a link. Then, goat-PAP (130157 Dako) at a dilution of 1:100 was used before the addition of Diaminobenzidine (DAB) to induce colour formation.

Statistical analysis: Hormone values were analyzed by using analysis of variance and General Linear Model procedures in the Statistical Analysis System (SAS, 1999) for treatments and time effects as well as interactions between the variables. Differences between means of significant effects were separated by the probability of differences procedure of SAS (1999).

RESULTS

Experiment 1: Effects of chlorpromazine or cyproheptadine on broodiness and plasma PRL and LH concentrations

Changes in broody behaviour: The control hens continued to nest throughout the period when they were broody. Out of twenty chlorpromazine treated hens, sixteen (80%) terminated broodiness on 4.6±0.8 days after start of treatment, while four (20%) continued to be broody even after 7 days of treatment. Again, out of twenty cyproheptadine treated hens, thirteen (65%) terminated broodiness on 2.3±0.2 days, while seven (35%) continued to be broody and showed persistent nesting after 7 days of treatment.

Changes in plasma PRL levels: The treatment of Yuehuang hens with 5-HT receptor antagonist (cyproheptadine) and dopamine receptor antagonist (chlorpromazine) significantly ($p<0.5$) depressed the release of PRL and disrupted broodiness (Table 1). In the control hens, there were no significant ($p>0.5$) changes in plasma PRL levels throughout the blood sampling period. The plasma PRL levels were maintained between 200 and about 400 ng/ml. There were no significant ($p>0.05$) differences in plasma PRL levels between the three groups before the drug treatment started for groups 1 and 2. Following drug treatment, the hens which terminated broodiness had plasma PRL levels significantly ($p<0.01$) lower than those in the control group (Table 1).

Changes in plasma LH levels: For the hens which terminated broodiness by treatment with chlorpromazine or cyproheptadine, plasma LH levels significantly ($p<0.01$) increased from below 1 ng/ml before drug treatment to almost 3 ng/ml, on day 19 following onset of broodiness, whereas the levels in control hens was maintained below 1 ng/ml throughout the sampling period (Table 2).

Table 1: Plasma prolactin concentrations (ng/mL) of chlorpromazine treated (group 1) and cyproheptadine treated (group 2) hens which terminated broodiness following drug treatment and control hens (group 3)

Days of broodiness	2	5	9	14	19
	Plasma prolactin concentrations (ng/mL) (Mean±SEM)				
Group 1	130.78±27.27b	13.71±4.18a	8.98±3.61a	18.77±9.71a	45.50±29.26a
Group 2	215.24±54.02b	3.95±0.79a	5.32±1.02a	10.46±0.99a	26.32±04.70a
Group 3	211.02±28.59b	262.88±45.71b	361.46±39.25b	254.29±61.16b	212.76±58.52b

Values with different letters either vertically or horizontally indicate significant difference ($p<0.01$)

Table 2: Plasma LH concentrations (ng/mL) of control hens (group 3, n = 7), chlopromazine treated (group 1, n = 7) and cyproheptadine treated (group 2, n = 6) which also terminated broodiness following drug treatment

Days of broodiness	2	5	9	14	19
	Plasma LH concentrations (ng/mL) (Mean±SEM)				
Group 1	0.70±0.23b	1.59±0.34a	1.65±0.28a	2.43±0.46a	3.00±0.42a
Group 2	0.62±0.18b	1.56±0.22a	1.71±0.20a	2.49±0.34a	2.75±0.34a
Group 3	0.71±0.16b	0.62±0.19b	0.66±0.09b	0.82±0.09b	0.88±0.12b

Values with different letters either vertically or horizontally indicate significant difference ($p<0.05$)

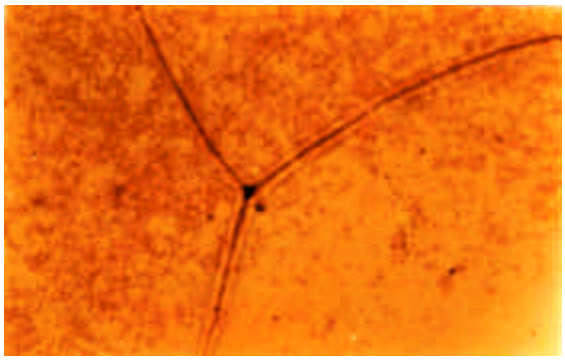

Plate 1: Non-treated control hens killed on day 2 after onset of broodiness (x100)

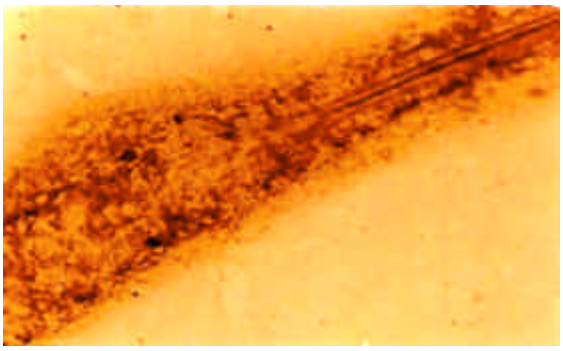

Plate 2: Non-treated control hens killed on day 7 after onset of broodiness (x100)

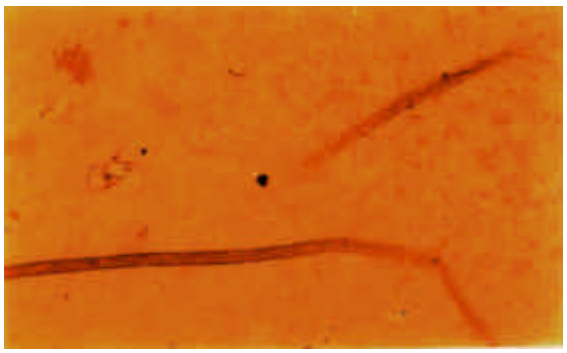

Plate 3: Cyproheptadine treated hens which terminated broodiness and were killed on day 7 (x100)

Experiment 2: Observations on VIP neurons in the hypothalamus: A higher number of positive VIP neurons were found in the hypothalamus of the control hens on days 2 and 7 of broodiness. The neurons were strongly stained and also some areas outside the neurons were stained as well (Plates 1 and 2). For hens which terminated broodiness by either chlopromazine or cyproheptadine treatment, VIP positive neurons were not strongly stained nor was there a strong VIP positive background in the vicinity of the neurons (Plates 3 and 4). These results indicate that in broody hens, there is a higher production of VIP by the hypothalamus than in hens in which drugs are used to terminate broodiness.

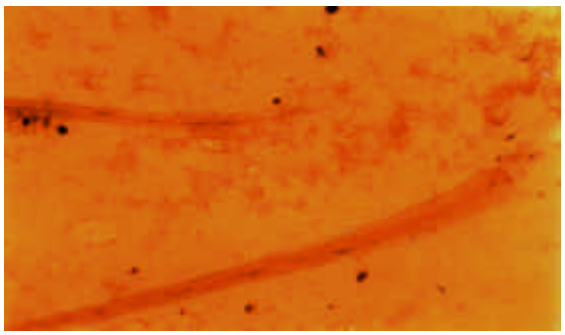

Plate 4: Chlopromazine-treated hen which terminated broodiness and were killed on day 7 (x100)

DISCUSSION

The dopamine receptor antagonist, chlopromazine and serotonin (5-HT) receptor antagonist, cyproheptadine, were very effective in blocking the onset of broodiness when they were given at the right time. The hens were treated starting from the second day after the onset of broodiness so the drugs were very effective in interfering with the functional activities of neurons containing dopamine and 5-HT to block them from stimulating VIP to release PRL. As a result, the plasma PRL levels were depressed so broodiness was disrupted and the hens deserted their nests. In broody turkey hens, dopaminergic inhibition diminishes and allows for increased PRL release (El Halawani *et al*., 1991a,b). However, when chlopromazine was administered to the broody hens, it was very effective in blocking the functional activity of dopamine in the hypothalamus to inhibit PRL release. This may be the reason why broodiness was terminated in eighty percent (80%) of the treated hens. Dopamine inhibits PRL release from chicken and turkey anterior pituitary cells *in vitro* (Harvey *et al*., 1982). This indicates that the stimulatory effect of dopamine occurs in the hypothalamus. Increased dopaminergic activity could stimulate PRL synthesis and release which in turn have an anti-gonadal effect by blocking gonadotropin stimulation (Millam *et al*., 1980) to cause broodiness in birds. Therefore, the use of dopamine receptor antagonist inhibits the stimulatory effect of increased dopaminergic activities to block PRL release. This in turn disrupts broodiness to maintain egg laying. There is a transitory increase in the metabolism of dopamine in the brain of turkey hens during transition from laying to the broody phase of the reproductive cycle (El Halawani and Burke, 1976). This increase corresponds with the period when plasma PRL

is increasing and plasma LH is decreasing (Burke and Dennison, 1980). These observations indicate that dopamine regulates PRL release in birds and plays a causal role in broodiness. It is therefore possible to use dopamine receptor antagonist, chlopromazine, to inhibit the regulatory effect of dopamine to block PRL release and to disrupt broodiness in domestic hens.

Cyproheptadine, the 5-HT receptor antagonist, might have entered the hypothalamus to inhibit the functional activity of 5-HT to block the PRL-releasing effect of 5-HT. As a result, sixty five percent (65%) of the drug treated hens terminated broodiness. Serotonin (5-HT) stimulates PRL release in avian species (Hall *et al*., 1986). However, the failure of 5-HT to induce PRL release *in vitro* (Proudman and Opel, 1981; Fehrer *et al*., 1985) and also the absence of 5-HT receptors in the anterior pituitary of bantam hens (Macnamee and Sharp, 1989a) indicate that the excitatory effects of 5-HT on PRL release may occur within the hypothalamus. This demonstrates that the functional integrity of serotonergic neurons is involved in the mechanism of PRL release in the hypothalamus. It is therefore possible that cyproheptadine might have blocked the functional activity of serotonergic neurons in the hypothalamus to inhibit the stimulation of VIP to release PRL so as to terminate broodiness in sixty five percent (65%) of the treated hens. This substantiates the proposition that 5-HT is a central neurotransmitter that stimulates PRL secretion (Hall *et al*., 1986). The disruption of broody behaviour in the hens by oral administration of dopamine and 5-HT receptor antagonists is consistent with the view that an increased concentration of plasma PRL maintains broody behaviour (Janik and Buntin, 1985; Sharp *et al*., 1988). Therefore, in the presence of all environmental stimuli necessary for the maintenance of broody behaviour, the depression of plasma PRL after the administration of the drugs to inhibit the functional activities of dopamine and 5-HT was associated with the disruption of broodiness. This demonstrates that 5-HT and dopamine may be potential PRL regulators in domestic hens. Again, it indicates that the hypothalamic control of PRL secretion in birds is primarily under stimulatory control, in contrast to the inhibitory control exerted by dopamine in mammals (Hall *et al*., 1986). The neural mechanisms involved may have been fully activated in the broody hens since in all species studied, the level of plasma PRL is increased during the broody period (Goldsmith, 1983). This is consistent with the finding that hypothalamic 5-HT induces PRL release indirectly by stimulating VIP release which in turn acts directly on the anterior pituitary gland (Macnamee and Sharp, 1989a; El Halawani *et al*., 1990). The observation that twenty (20%) and thirty five percent chlopromazine and cyproheptadine treated hens respectively did not terminate broodiness may be due to individual variations in the response to the drug treatment, or drug absorption. Disruption of nesting is associated with drug treatment designed to disrupt broodiness. A broody hen which spends a lot of time on the nest is restricted from feeding and persistent nesting itself induces broodiness. Therefore, treating broody hens with chlopromazine and cyproheptadine starting from the second day after the onset of broodiness tends to thwart the hens desire to nest so as to keep them reproductively active.

The observations of different numbers of VIP neurons in the hypothalamus of the control hens and the drug treated hens which terminated broodiness and were killed on day 7 after the onset of broodiness correspond to the different levels of circulating PRL in the birds during the different periods. The relationship between changes in the VIP contents and alterations in PRL in the birds during the different stages in the reproductive cycle indicates that fluctuations in hypothalamic VIP play a major role in the regulation of PRL secretion. This shows that VIP is a potent PRL-releasing neuropeptide in domestic hens. In the drug treated hens which terminated broodiness and were killed on day 7, it could be said that PRL release was probably depressed for about 7 days when the drugs were present in the peripheral circulation. This observation shows that VIP may be the only PRL-releasing factor maintaining high levels of plasma PRL.

The absence of VIP neurons in the hypothalamus of the chlopromazine and cyproheptadine treated hens which terminated broodiness and were killed on day 15 after onset of broodiness shows that there was marked decline in the number of VIP neurons in the hypothalamus and this was associated with a significant depression in circulating PRL. This is consistent with the hypothesis that changes in plasma PRL levels in broody hens are associated with changes in hypothalamic VIP. Therefore, reduced levels of PRL in the drug treated broody hens were associated with reduction of VIP in the hypothalamus. These results indicate that chlopromazine and cyproheptadine can inhibit PRL release and the effect is through inhibition of VIP in the hypothalamus.

Conclusion: The reproductive efficiency of domestic hens can be improved by the use of chlopromazine and cyproheptadine which inhibit the functional activities of dopamine and serotonin and thereby inhibit PRL release to disrupt broodiness in domestic hens to maintain egg production. Again, the results indicate that VIP is an important PRL releasing neuropeptide in domestic hens.

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