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INTRODUCTION

Feeding behavior is important for survival. The decision of whether to eat is controlled by the brain, and is dependent on the current energy supply from the gut, competition from other drives, and previous experience with the food in question. Feeding terminates when a satiating quantity of food has been eaten, thus being detected by the brain. The physiological control of feeding involves neural and chemical signals that are integrated by the central nervous system (CNS). Feeding provides all of the body’s macronutrients (carbohydrates, lipids, and proteins) and micronutrients (vitamins and minerals) necessary for maintenance and production. It is also a fundamental aspect of energy homeostasis in all animals.

Modulation of feeding behavior is a normal part of survival, and may be influenced by many internal and external factors. Internal factors include palatability of food, maintenance of energy balance, nutrient specific appetites, and gut-nervous system interactions. External factors may be attributed to conditions of dietary nutrients, such as proteins and amino acids (Mercer et al. 1990). Other external factors such as adverse housing, weather, disease, sanitary conditions, or imbalances in dietary nutrients may also induce suppression of food intake. In addition, pathological conditions may interrupt or hinder regulatory aspects of feeding behavior leading to inappropriate feed intake.

Neurochemical control of food intake has only been investigated since the early 1980s. Several studies have been conducted using various neurotransmitters involved in food intake regulation. For example, ICV injections of epinephrine into the lateral ventricle increased food intake in broiler-type chickens (Denbow et al. 1981). It is important to understand feed regulation in order to develop methods to increase consumption in market broilers and turkeys, or to restrict intake to control obesity of breeders.
Neural Regulation of Food Intake in Chickens

Food intake regulation is a complex system involving both peripheral and central sites of control. Hypotheses proposed to explain the neural regulation of food intake in birds stem largely from research conducted using mammals. Early hypotheses explaining the neural regulation of food intake were developed by Stellar (1954) after the discovery of Anand and Brobeck (1951) who found that lesioning the lateral area of the hypothalamus of the rat caused decreased food intake. Later studies verified that bilateral lesioning of the lateral hypothalamus (LHA) produced aphagia and body weight loss (Feldman et al. 1957; Leibowitz et al. 1981). Earlier, Hetherington and Ranson (1940) demonstrated that electric lesioning of the ventromedial hypothalamic nucleus (VMH) of the cat caused overeating and obesity. The VMH, which was termed the “satiety center”, contained a set of neurons that projected to, and inhibited the LHA, the “feeding center” (Kuenzel, 1994).

This dual hypothesis theory has predominated for a long time. However, evidence shows that this view may be too restrictive and that many neural pathways transverse the hypothalamus. Gold (1973), using hypothalamic knife cuts which spared the VMH of rats, still induced obesity. Fiber tracts involved in hyperphagia, therefore, appeared to involve a longitudinal neural pathway, rather a direct fiber projection between the VMH and LHA (Sclafani and Grossman, 1969; Gold, 1973). Sclafani and Kirchgessner (1986) discovered that hyperphagia did not result from the traditional lesioning of the VMH, but instead from the interruption of pathways originating from the paraventricular (PVN), traversing the medial hypothalamic area for the nucleus tractus solitarius (NTS) and the dorsal motor nucleus of the vagus. This indicated that the control of food intake involved neural circuits within the central nervous system rather than circumscribed sites. Other neural sites known to be important for regulating food intake in mammals include the PVN, NTS, and parabrachial nucleus (Kuenzel, 1994). The PVN is a critical structure that is very sensitive to noradrenergic stimulation of food intake (Leibowitz 1978a, 1978b). Leibowitz et al. (1981) also found that lesioning the PVN, as well as the VMH, caused hyperphagia and obesity in the rat. Bilateral lesions of the dorsolateral parabrachial nucleus also caused hyperphagia and obesity (Nagai et al., 1987). Interestingly, a major
projection to the core of the VMH originates from the dorsolateral parabrachial region (Zaborsky et al., 1984).

Consistent with mammalian studies, electrolytic lesions of the VMH of White Leghorn chickens caused hyperphagia and obesity (Lepkovsky and Yasuda, 1966; Snapir et al., 1973; Sonoda, 1983). However, there are no published studies on the effects of PVN or parabrachial nucleus lesions in any avian species. The large size of the PVN, and its connections to the NTS and dorsal motor nucleus of the vagus (Arends et al., 1988), parabrachial nucleus (Wild et al., 1990), and the median eminence (Korf, 1984) warrants the study of its effect on food intake. There are also no published studies comparing lesions of the VMH to the PVN in birds determining if there was metabolic vs. regulatory obesity. Sonada (1983) demonstrated that insulin levels were higher in VMH lesioned chickens than controls.

Although there are several similarities between birds and mammals in the control of food intake, there are also noticeable differences. Lateral hypothalamic lesions have been performed in chickens and resulted in reduced food intake (Feldman et al., 1957). However, whereas such lesions caused prolonged hypophagia in mammals, the response in birds appears very transient. Kuenzel (1982) observed that bilateral lesions involving the ansa lenticularis, quintofrontal tract and lateral hypothalamus were most effective in reducing food intake, but consumption returned to normal within 1 week.

Kuenzel (1989) reviewed five neural pathways having specific roles in the control of food intake in birds. These pathways included the trigeminal sensorimotor system, the visual system/basal ganglia pathway, the gustatory system, the olfactory pathway and the autonomic nervous/parasympathetic pathway. The best understood of these pathways is the trigeminal system. The trigeminal system begins with sensory nerves innervating the upper and lower mandibles and buccal cavity, and terminates with nerves projecting to jaw muscles. This circuit receives sensory input from, and sends efferent fibers to, the beak and its controlling muscles (Denbow, 1994). This pathway controls mandibulation and grasping of seeds and pellets. The visual system/basal ganglia pathway includes both the tectofugal and thalamofugal pathways, and interacts with the avian paleostriatal complex, which is equivalent to the mammalian basal ganglia. This pathway is involved in the recognition of food, as well as in orienting the body with respect to its position in three-dimensional space. The gustatory system is involved with taste. It receives input from the glosso pharyngeal and chorda tympani branch of the facial
nerves. The olfactory system is composed of more than one pathway. Robinzon et al. (1977) observed that the removal of the olfactory bulbs of chickens and red-winged blackbirds caused hyperphagia without obesity. The final pathway, the autonomic/parasympathetic pathway, is involved in the interaction of the hypothalamus and the dorsal motor nucleus of the vagus.

The preoptic region, as well as the anterior and lateral hypothalamus, are known to be sites involved in the control of water intake in birds. Scott and Van Tienhoven (1974) demonstrated the earliest results on the regulation of water intake in birds. Electrical stimulation or lesioning specific areas of the avian brain showed that hyperstriatal lesions did not affect water or food intake. Since then, interest has focused on the hypothalamus. Polyuria and polydipsia were produced in chickens by electrolytic lesions of the nucleus supraopticus or the hypothalamo-hypophysial tract plus the tuberomammillary nucleus (de Caro and Massi, 1983). Currently, more extended and detailed investigations are needed for a complete description of the brain regions and neural pathways regulating water intake in chickens.

Neurochemical studies into the control of food intake have only been conducted during the last two decades. The first compounds given centrally to birds were biogenic amines. Denbow et al. (1981) showed that ICV injections of epinephrine in broilers increased food intake, while dopamine and norepinephrine had no effect. However, no significant change in food intake was noted in Leghorn chicks given epinephrine ICV (Denbow et al., 1981). When 6-hydroxydopamine was given ICV to broiler chicks, food intake was greatly reduced as was striatal, hypothalamic, and brainstem norepinephrine and striatal dopamine (Kuenzel et al., 1987b).

Several peptides, amino acids, and hormones have also been shown to stimulate food intake. Within the opioid peptide family, ICV injections of $\beta$-endorphin (ostrich) was first shown to stimulate food intake (Deviche and Schepers, 1984). Methionine-enkephalin stimulated food intake in broiler-type and egg layer type chicks (McCormack and Denbow, 1988). Within the polypeptide family, neuropeptide Y and neuropeptide YY, given ICV to broiler chicks, increased food intake (Kuenzel et al., 1987a; Kuenzel and McMurtry, 1988). Avian pancreatic polypeptide given ICV to Leghorn chickens also increased food intake (Denbow et al., 1988). Cholecystokinin-8 given ICV to broilers decreased food intake (Denbow and Myers, 1982).
Central Nervous Control of Body Temperature in the Fowl

When environmental temperature is elevated above thermoneutrality, chickens pant vigorously and lift their wings to facilitate heat loss, whereas in cold environments birds shiver and fluff their feathers to conserve heat. These behaviors are indicators for heat-loss or heat-production activities.

In the fowl, important thermoregulatory structures reside in the anterior hypothalamic and preoptic regions, along with the midbrain, medulla, and spinal cord (Kanematsu, 1983). Feldman et al. (1957) reported disturbances in temperature regulation following bilateral electrolytic lesions in the diencephalon. The maximum body temperature recorded was 43.5°C in these chickens. Yamauchi and Yasuda (1978) observed hyperthermia in chickens following bilateral lesions in the area ranging from the anterior commissure to approximately 480 µm rostral to it in the lateral hypothalamus.

Kanematsu (1982) observed that panting or polypnea in chickens was elicited by electrical stimulation of the preoptic-anterior hypothalamus, especially the post medial ventral area to the anterior commissure. Shivering was elicited by electrical stimulation of the medial hypothalamic area ventrocaudal to the anterior commissure, while destruction of this area resulted in a loss of the ability to maintain body temperature against the cold (Kanematsu, 1982).

Several studies have been conducted using acetylcholine, monoamines, neuropeptides, and prostaglandins to investigate central nervous system control of thermoregulation. Scott and van Tienhoven (1974) observed that ICV injections of norepinephrine, epinephrine and dopamine caused hyperthermia or inhibited heat-production behavior at 10°C, but was not effective at an ambient temperature of 30°C in non-laying hens. Intrahypothalamic injections of acetylcholine (Ach) or physostigmine (Phy) did not produce thermoregulatory effects. However, in combination (Ach 5-20 µg + Phy 5 µg), they inhibited shivering and induced a fall in body temperature of pigeons (Pyornila et al., 1977). Pyornila et al. (1977) also observed that intrahypothalamic injections of carbamylcholine (Cch) produced cessation of shivering and led to a marked fall in body temperature and oxygen consumption. Intraventricular injections of β-endorphins produced a fall in rectal temperature of unanesthetized rats at ambient temperatures of 8°C and 22°C, but produced no change at 30°C (Lin et al., 1979). Microinjections of bombesin in or near the preoptic area caused hypothermia, whereas injections into other forebrain and
midbrain areas failed to lower body temperature in rats (Pittman and Tache, 1980). Prostaglandins (PG) PGE₂ and PGA₁, injected into the hypothalamus, induced sedation, sleep and hyperthermia in hens while PGF₂ induced hypothermia (Nistico and Marley, 1973; Kane and Peterson, 1975).

**Histamine as an inhibitory neurotransmitter of food intake**

Histidine, an indispensable amino acid, is the biosynthetic precursor of the neurotransmitter histamine (HA). Systemic histamine is transported through the blood brain barrier via a carrier mediated transport system for large neutral amino acids (Mercer et al., 1990). In the hypothalamus, histidine is decarboxylated to HA by histidine decarboxylase in a restricted area located in the tuberomammillary nucleus of the posterior hypothalamus (Leurs and Timmerman, 1997). A rise in serum histidine, coupled with a reduction of the other indispensable amino acids as seen in protein malnutrition, has a tendency to raise brain histidine levels at the expense of other neurotransmitter precursors including tryptophan, phenylalanine, and tyrosine. Increased histidine transport leads to an increased rate of HA synthesis because central nervous system histidine decarboxylase is not saturated at normal physiologic concentrations of histidine (Hegstrand and Simon, 1985). The suprachiasmatic nucleus (SCN) and mesencephalic trigeminal sensory nucleus (Me5) were also found to be richly innervated with HA neurons (Itowi et al., 1988; Fujise et al., 1998). HA neurons in the Me5 modulate masticatory functions, especially eating speed through the mesencephalic motor nucleus. Brain HA is known to consist of two different sources. Histamine neuron systems project their efferent varicose fibers to almost all areas of the brain, with the hypothalamus being one the richest areas. Afferent fibers to the nucleus are mainly from the same limbic regions (Ericson et al., 1991).

Three histaminergic receptors have been identified in the CNS including H₁, H₂, and H₃. Both the H₁ and H₂ receptor belong to a large family of G-protein coupled receptors (GPCRs) (Leurs and Timmerman, 1997). The HA H₁ receptor is associated with the phospholipase C-catalyzed formation of inositol 1,4,5-triphosphate (IP₃) and 1,2-diacylglycerol (DAG). Histamine promotes production of IP₃ in several tissues including the brain via a pertussis toxin-insensitive G-protein. However, the actual nature of this G protein is still unclear (Leurs and Timmerman, 1997). The histamine H₂ receptor is coupled to the adenylate cyclase (AC) system. The histamine H₃ receptor, is thought to belong to a superfamily of G-protein coupled receptors.
One known central effect of hypothalamic HA and H₁ and H₂ agonists is induced release of corticotropin releasing factor (CRF), adrenocorticotropic hormone (ACTH), and corticosterone (Mercer et al., 1990). Histaminergic neurons have been demonstrated to suppress food intake through H₁-receptors in the VMH and PVN. H₁ and H₂ receptors are activated at this time causing neurochemical changes in the corticotropin releasing factor (CRF) neuron, thus producing several brain functions in discrete nuclei related to food intake such as the ventromedial nucleus and (VMH) paraventricular nucleus (PVN) (Ookuma et al., 1988, 1993; Mercer et al., 1990). Alterations in brain histamine concentration are also associated with changes in feeding, drinking, neuroendocrine secretion, sleep-wakefulness cycle, thermoregulation, and pain threshold (Doi et al., 1994). Hypothalamic neuronal histamine controls several adaptive behaviors including decreased feeding and ambulation, and an increase in water intake to regulate body temperature (Sakata et al., 1997). Ookuma et al. (1988) demonstrated that neuronal histamine and H₁-receptors in the preoptic area (POAH) are known to be related to thermoregulation since microinjection of exogenous histamine lowered body temperature, and this response was blocked by H₁-antagonists. As with many other neurotransmitters systems, a presynaptic receptor for HA H₃ exists and regulates the release and synthesis of histamine (autoreceptor). Exogenous HA is thought to inhibit release of HA through these autoreceptors (Timmers and Leurs, 1997; Arrang et al., 1983, 1987). However, the actions of H₃-receptors are not clear.

Histamine blockade by histaminergic antagonists has been shown to stimulate appetite, and the suppressive action of HA on food intake could be blocked by pretreatment with antihistamines (Orthen-Gambill, 1988). Several studies show that infusion of H₁-receptor antagonists, but not H₂-receptor antagonists, into the third cerebroventricle significantly increased feeding (Ookuma et al., 1988; Lecklin and Tuomisto, 1998; Sakata et al., 1988a,b, 1990; Doi et al., 1994; Machidori et al., 1992). H₁-receptor antagonists easily penetrate the brain and are very useful for in vivo studies. In rats, the HA H₁ antagonist chlorpheneramine maleate induced feeding in a dose-related manner after infusion in the third cerebroventricle (Fukagawa et al. 1989; Sakata et al. 1988a), while iv administration of H₁-antagonists did not elicit feeding, therefore verifying that feeding was induced by H₁ antagonists acting in the CNS, and not through secondary peripheral effects. Furthermore, food intake decreases when the release of
endogenous HA is stimulated by ICV application of thioperamide, an H₃ receptor antagonist, which blocks the autoinhibition of HA release (Machidori et al., 1992).

**Bombesin and Food Intake**

Bombesin (BM) is a tetradecapeptide derived from amphibians (Chronwall et al., 1985; Moody et al., 1986). It is located in discrete forebrain and hindbrain sites such as paraventricular nucleus (PVN), the nucleus tractus solitarius (NTS), interpeduncular nucleus, central grey, dorsolateral tegmental nucleus, dorsoparabrachial nucleus, nucleus of the solitary tract and trigeminal complex (Panula et al., 1982). The highest concentration of BM fibers is found in the hypothalamus (Merali and Banks, 1994; Plamondon and Merali, 1997). Intense immunoreactivity was also found in superficial layers of the posterior horn of the spinal cord (Panula et al., 1982).

Administration of BM suppresses food intake in diverse species such as rats (Flynn, 1989) and turkeys (Denbow, 1988). Kyrokouli et al. (1987), using brain microinjections and lesioning, revealed that certain hypothalamic and hindbrain structures such as the PVN and nucleus solitari are particularly sensitive to BM-induced hypophagia. Interestingly, BM decreases water intake in rats when food was available (Flynn, 1989). However, when food was removed, there was no effect on food intake (Flynn, 1989). These results indicate that BM can act directly on caudal brain sites to inhibit food intake. Central or systemic administration of BM has a number of other biologically significant actions including elevation of blood glucose (Brown et al., 1979; Plamondon and Merali, 1993) and elicitation of grooming (Gmerek and Cowan, 1983; Pert et al., 1980) and locomotor activity (Merali et al. 1994).

Systemically administered BM produces a satiety-like effect in lean and genetically obese mice (McLaughlin and Baile, 1981; Taylor et al., 1985), hamsters (Miceli and Malsbury, 1985), and turkeys (Denbow, 1988). In addition, iv infusions of BM caused dose-related inhibitions of test meals in baboons (Woods et al., 1988). Stuckey et al. (1985) found that the total neural disconnection of the gut from brain abolished feeding suppressant effects of systemically administered BM. This suggests that there is a peripheral site of action for systemically administered BM. Merali et al. (1993) suggested that the effect of systemically administered BM is neurally communicated to the brain where BM receptors in the caudal brain stem sites are involved in the suppression of food intake.
There is anatomical evidence showing overlap between BM and HA neurons leading to the hypothesis that BM may mediate its effects not only through CRF, but also through activation of the histaminergic fibers. When alpha methyl histamine ($\alpha$-MH) and Imetit, which are both $H_3$ agonists, were infused into the third cerebroventricle of rats, the satiating effects of BM were blocked (Merali and Banks, 1994; Kent et al., 1997). When $\alpha$-MH was administered alone, it failed to significantly affect food intake. In addition, thiopramide, an $H_3$ receptor antagonist, blocked the effects of Imetit. Therefore, it appears that BM affects food intake by facilitating histaminergic activity at sites which have not been determined, and that $H_3$ agonists attenuate its effect on food intake by inhibiting the release of HA (Merali and Banks, 1994; Kent et al., 1997).

**Corticotropin Releasing Hormone and Food intake**

Corticotropin-releasing hormone (CRH) is a 41-amino polypeptide (Brown et al. 1985; Lavicky and Dunn, 1993). It is synthesized from a precursor of 196 amino acids. Neurons, which secrete CRH, are found in the anterior portion of the paraventricular nuclei just lateral to the thyroid releasing hormone (TRH)-secreting neurons. It has nerve endings that are found in all parts of the external layer of the median eminence. CRH has been reported to be a putative neurotransmitter and/or neuromodulator within the CNS (Emoto et al., 1993). It is released into the hypophyseal portal blood after stress and it stimulates ACTH release (Brown and Fisher, 1985). The exact CNS site of action of CRH, which increases plasma concentrations of catecholamines, has not yet been determined. According to Spinedi et al. (1988), central monaminergic systems play an important role in the regulation of CRH in ACTH secretion.

Results suggest that central histamine decreased food intake through the activity of the CRF neuron on the PVN (Morley 1989). Central administration of CRF decreases food intake in chickens (Denbow et al., 1999, in press) and rats (Britton et al., 1982; Morley and Levine, 1982). CRF has been reported to suppress feeding when microinjected into the PVN, but not LHA or VMH, globus pallidus or corpus striatum. One known central effect of hypothalamic HA and $H_1$ and $H_2$ agonists is to cause the release of corticotropin releasing factor (CRF), therefore suggesting that the blockade of these histaminergic receptors may block the release of CRF.

Studies report that autonomic and behavioral syndromes that are elicited by CRF strongly resemble that of BM, and that there is a significant anatomical overlap in the localization of these
two neuropeptides (Panula et al., 1982). Hale et al. (1984) concluded that gastrin releasing peptide (GRP) was shown to potentiate ovine CRF stimulated ACTH release from isolated rat pituitary. Familiari et al. (1988) observed that BM consistently potentiated the release of ACTH in response to CRF pulses. This suggested that BM potentiates CRF-stimulated ACTH release from acutely perfused rat pituitary cells and that this potentiation of CRF by BM is glucocorticoid dependent.

An interaction between these two peptides is further supported by studies demonstrating that many of the autonomic and endocrine effects of exogenous BM are blocked by central pretreatment with the CRF antagonist, αCRF (Merali et al., 1994). Plamondon and Merali (1997) also demonstrated that CRF mediated the anorexigenic effects of BM. Central administration of two CRF antagonists blocked the behavioral and feeding suppressant actions of BM. However, administration of αCRF alone failed to affect food intake. Blockade of central CRF receptors prevented BM’s suppressant action on feeding suggesting that BM may elicit its effects on feeding and related behaviors through the release of central CRF pathways (Plamondon and Merali, 1997; Garrida et al., 1998). The precise receptor-based mechanism by which CRF and BM may interact, however, remains unknown.

In time, as more research is conducted using substrates that alter feeding, it may be possible to manipulate these systems. The purpose of these studies were to observe the effects of centrally administered neurotransmitters on food intake and thermoregulation.
REFERENCES


CHAPTER ONE
FEEDING, DRINKING AND TEMPERATURE RESPONSES TO
INTRACEREBROVENTRICULAR HISTAMINE

ABSTRACT

Histamine (HA) has been shown to alter several biological functions in rats and other mammals. The present studies examine the effects of intracerebroventricular (ICV) injections of HA and two HA antagonists, the H₁ receptor antagonist chloropheneramine maleate (CM), and the H₂ receptor antagonist cimetidine (CIM), on food and water consumption and body temperature in birds. Single Comb White Leghorns (SCWL) and broiler cockerels were utilized for these experiments. A 23-gauge thin-walled stainless steel cannula was stereotaxically implanted into the right lateral ventricle. Each type of bird received the same treatments. The first pair of experiments consisted of ICV injections of HA and its effects on food and water consumption. Histamine was infused at dosages of 0, 25, 50, and 100 µg/10 µl of artificial cerebrospinal fluid. Histamine significantly decreased food and water intake (P ≤ 0.05) in a dose-dependent manner. The second pair of experiments examined the effects of HA on water intake while birds had no access to feed. Water intake was not significantly affected by ICV injections of HA. The next pair of experiments examined the effects of HA on body temperature. In SCWL, body temperature was not affected by HA until 165 min post-injection when HA decreased temperature in a quadratic dose response with maximum hypothermia being achieved at a dose of 25 µg. In contrast, HA increased body temperature in broilers beginning at 75 min post-injection. In the final series of experiments, the anorexia induced by HA was attenuated in SCWL and broilers with pretreatment of either CM or CIM. These results suggest that HA has an anorexigenic effect in SCWL and broiler cockerels, and this effect is mediated by both H₁ and H₂ receptors. Water intake is not directly affected by the ICV injection of HA. Whereas, HA increased body temperature in broilers, the response in SCWL is equivocal.

Key words: Histamine, Food intake, Water intake, Chloropheneramine maleate, Cimetidine, Chickens
INTRODUCTION

Histamine (HA) is a putative neurotransmitter that is heterogeneously distributed in the brain. It is synthesized from the amino acid precursor histidine, which is then decarboxylated to HA via histidine decarboxylase, most prominently in the tuberomammilary nucleus of the posterior hypothalamus (Leurs and Timmerman, 1997). HA localized within the brain must be synthesized via this pathway since peripheral HA does not cross the blood brain-barrier (Mercer et al., 1994). Increases in plasma histidin concentrations relative to those of other amino acids transported by the large neutral amino acid carrier result in an increased transport of histidine into the brain. This increased histidine transport leads to an increased rate of HA synthesis because central nervous system histidine decarboxylase is not saturated at normal physiologic concentrations of histidine (Hegstrand and Simon, 1985).

There are three central HA receptors, H₁, H₂, and H₃, that are involved in regulatory processes such as food intake, sleep-wakefulness, and thermoregulation (Leurs and Timmerman, 1997). Histamine acts within the central nervous system to suppress food intake through H₁ receptors in rats (Sakata et al., 1988a,b; Fukagawa et al., 1989; Ookuma et al., 1989; Machidori et al., 1992) and goats (Tuomisto and Eriksson, 1979). Feeding was induced by injection of histamine H₁, but not H₂-antagonists in the ventromedial hypothalamus (VMH) (Sakata et al., 1988a; Ookuma et al., 1989; Mercer et al., 1994). Itowi et al. (1988) examined the effect of HA infused into the suprachiasmatic nucleus (SCN), where it also decreased food intake in the rat.

Histaminergic activity is reduced by α-fluromethylhistidine (FMH), a “suicide” inhibitor of histidine decarboxylase. Daily food intake was increased in rats given (60µg/day) FMH ICV with an osmotic minipump or when given acutely into either the third ventricle, PVN or VMH (Sakata et al., 1990; Tuomisto et al., 1994). The suppressive effect of HA is also attenuated by the pretreatment of FMH (Sakata et al.1988b; Fukagawa et al. 1989).

Hypothermia was induced by the infusion of HA in rats acting at both H₁ and H₂ receptors in rats (Clark et al., 1980; Khan and Gupta, 1986; Chen et al., 1995). Injections of H₁ and H₂ antagonists abolished histamine hypothermia. Intracerebroventricular injections of thioperamide, a histamine H₃ antagonist, caused hypothermia (Chen et al., 1995).

The present study was conducted to determine the effects of exogenous histamine on food and water intake and thermoregulation in broiler and Single Comb White Leghorns.
Furthermore, the receptor subtypes whereby histamine modulates these behaviors was investigated.
MATERIALS AND METHODS

Animals. Broiler and Single Comb White Leghorn (SCWL) cockerels were reared in heated batteries with continuous lighting until 4 and 7 weeks of age, respectively. Birds were provided a mash diet (20% crude protein, 3.0% crude fat, and 2864 kcal/kg of metabolizable energy) and water for ad libitum consumption. At approximately 3 and 6 weeks of age, the birds were transferred to individual cages measuring 17.6 x 26.4 x 17.6cm. Each caged was supplied with individual feeders and waterers.

Surgical preparation. At 4 and 7 weeks of age respectively, broilers and SCWL were anesthetized with sodium pentobarbital (25mg/kg body weight iv) and a 23-gauge thin-walled stainless steel guide cannula was stereotaxically implanted into the right lateral cerebral ventricle as described by Denbow et al (1981). The cannula was secured with 3 stainless steel screws placed in the calvaria surrounding each guide cannula and acrylic dental cement (Cranioplast Plastics Product Company, Roanoke, VA) was applied to the screws and guide cannula. Placement of the cannula into the ventricle was verified by the presence of cerebrospinal fluid. Birds were allowed a minimum of 3 days recovery prior to injection.

Experiment 1. This experiment evaluated the effect of ICV administered histamine (Sigma). Eight SCWL cockerels were used in a replicated Latin Square design in which birds and days were the blocking factors. All solutions were prepared in artificial cerebrospinal fluid (aCSF; Anderson and Heisey, 1972) which also served as a control. Birds were injected with 0, 25, 50, 100 µg of histamine in a volume of 10 µl. Injections of histamine were made using a 27-gauge stainless steel injection cannula connected to a 10µl Hamilton syringe with a 60 cm length of PE-20 tubing (Clay Adams). Food and water intake was monitored at 15-minute intervals through three hours postinjection.

Experiment 2. This experiment was similar to Experiment 1 except broilers were used instead of SCWL.

Experiment 3. To determine the effects of histamine on water consumption, water intake was monitored for 3 hr following the ICV injection of 0, 25, 50, and 100 µg of HA in a volume of 10 µl during which the birds did not have access to food. Water consumption was monitored at 15-minute intervals for three hours postinjection. The birds did not receive any food during this experiment.
Experiment 4. The experiment was similar to Experiment 3 except broilers were used instead of SCWL.

Experiment 5. To determine the effect of histamine on body temperature, thermistor probes were inserted into the colon, immobilized, and connected to a telethermometer (Model 46 TUC, Yellow Springs Instrument Co., Yellow Springs, OH). Using the same Latin square design as in Experiment 1, histamine was injected ICV and body temperature recorded every 15 minutes through 3 hours postinjection. Food was not available during the experiment.

Experiment 6. The experiment was similar to Experiment 5 except broilers were used instead of SCWL.

Experiment 7. This experiment evaluated the effect of ICV administered histamine, and a histamine \( H_1 \)-receptor antagonist chlorpheneramine maleate (CM) (Sigma). Eight SCWL cockerels were used in a replicated Latin square design in which birds and days were the blocking factors. All solutions were prepared in aCSF, which was also used as a control. Each bird received two injections. The first injection consisted of either 0 or 100 µg chloropheneramine maleate in a volume of 5µl. The second injection consisted of either 0 or 100 µg histamine in a volume of 5µl immediately after the first injection. Food and water intake was monitored at 15-minute intervals through three hours postinjection.

Experiment 8. The experiment was similar to Experiment 7 except broilers were used instead of SCWL.

Experiment 9. This experiment evaluated the effect of ICV administered histamine (Sigma), and a histamine \( H_2 \)-receptor antagonist cimetidine (Sigma). Using eight Leghorn cockerels in a replicated Latin Square design the experiment was similar to Experiment 7 except that the \( H_2 \)-receptor antagonist cimetidine (CIM) (Sigma) was used in place of CM.

Experiment 10. The experiment was similar to Experiment 9 except broilers were used instead of SCWL.

Analysis. In Experiments 1-6, cumulative food and water intake were analyzed using an analysis of variance at each time period. Treatment effects were separated into linear and quadratic contrasts to determine dose dependent responses at each time period. Significance implies to \( P \leq 0.05 \). In experiments 7-10, cumulative food and water intake were analyzed using an analysis of variance at each time period. Duncan’s multiple range test and orthogonal
contrasts were used for obtaining all pairwise comparisons and contrasts among sample means at each time period. Significance implies $P \leq 0.05$. 
RESULTS AND DISCUSSION

The food and water responses to intracerebroventricular (ICV) injections of histamine (HA) in SCWL and broilers are summarized in Figures 1 and 2, respectively. Food intake was decreased in both broilers and SCWL. The effect was dose dependent with 100µg HA being the most efficacious. Water intake was also decreased following ICV injections of HA in birds that had access to both food and water. When water intake was recorded in birds not allowed access to feed, there was no affect of HA on water consumption in SCWL (Figure 3) or broiler cockerels (Figure 4).

Orthen-Gambill (1988) reported that infusion of histidine decreased food intake in rats. A decrease in food intake was also observed when HA was injected into the lateral ventricle of cats (Clineschmidt and Lotti, 1973). These results, along with many others, show increased HA levels are associated with appetite suppression (Sakata et al., 1988a; Mercer et al., 1990; Doi et al., 1994). The highest concentration of HA is found in the VMH and PVN (Ookuma et al., 1989, 1993; Mercer et al., 1990). These areas are thought to play an inhibitory role in feeding behavior. Studies show that lesioning the PVN or VMH causes hyperphagia and obesity (Hetherington and Ranson, 1940). It is also noted that central administration of HA has an excitatory effect on VMH neurons (Schwartz, 1977). Thus, histamine appears to act by stimulating a site that inhibits feeding.

The food and water responses to ICV injections of HA and chlorpheneramine maleate (CM), an H₁-receptor antagonist, in SCWL and broilers are summarized in Figures 7 and 8, respectively. Injections of HA alone significantly decreased food intake (P≤ 0.05) in both SCWL and broilers. When birds were pretreated with CM, followed by HA, the suppressive action of HA on food intake was attenuated. There was no significant differences in water consumption in response to HA or CM in either SCWL or broilers. These results are consistent with previous studies showing that histamine decreased food intake acting at H₁ receptors in rats (Sakata et al., 1988a; Fukagawa et al., 1989). The site of action appeared to be the PVN and VMH (Ookuma et al., 1989; Sakata et al., 1988a).

The food and water intake responses for ICV injections of HA coupled with an H₂ antagonist cimetidine (CIM) are summarized in Figures 9 and 10. The anorexigenic effect of histamine was not seen in SCWL. However, when pretreated with cimetidine, there was an
increase in food intake over the control. In broilers, the decrease in food intake caused by ICV histamine was blocked by the \( \text{H}_2 \) antagonist cimetidine. This is opposite the response seen in rats, which orally administered cimetidine decreased food intake (Singh and Singh, 1995).

Temperature responses to ICV injections of HA are summarized for SCWL and broilers in Figure 5 and 6, respectively. Histamine produced hypothermia in SCWL at a dose of 25 \( \mu \text{g} \) with a quadratic trend at 165 min. However, histamine produced hyperthermia compared to the control in broiler cockerels.

Chen et al. (1995) observed a similar response in which a hypothermia was noted at low doses of HA. Similarly, it has been shown that ICV injections of HA resulted in hypothermia in rats (Sidman et al. 1974; Shaw 1971). Previous studies demonstrate that ICV injections of HA caused biphasic changes in body temperature in cats (Clark and Cumby 1976), mastomys (Dhawan et al. 1982), and guinea pigs (Khan and Gupta, 1986). However, broiler cockerels did not show the same response. Instead, there was a constant increase in temperature through 180 min post injection which was not proceeded by hypothermia.

The present experiments demonstrate that HA acts within the central nervous system to decrease food intake, while having no direct effect on water intake. Antagonism of HA with the \( \text{H}_1 \) and \( \text{H}_2 \) receptor antagonists CM and CIM attenuated the HA anorexigenic effects. Contrary to several studies in mammals, present experiments conclude that HA mediates anorexigenic effects through \( \text{H}_1 \) and \( \text{H}_2 \) receptors. Furthermore, it is concluded that HA causes hypothermia in SCWL and persistent hyperthermia in broiler cockerels.
Figure 1. Effect of intracerebroventricular injections of histamine (HA) on food (A) and water consumption (B) of Single Comb White Leghorn cockerels; artificial cerebrospinal fluid (aCSF); MIN, minutes; LIN, linear contrast; QUAD, quadratic contrast; PSE, pooled standard error, +, significant difference (P≤ 0.05)
Figure 2. Effect of intracerebroventricular injections of histamine (HA) on food (A) and water consumption (B) of broiler cockerels; artificial cerebrospinal fluid (aCSF); MIN, minutes; LIN, linear contrast; QUAD, quadratic contrast; PSE, pooled standard error, +, significant difference ($P \leq 0.05$).
Figure 3. Effect of intracerebroventricular injections of histamine (HA) on water consumption of Single Comb White Leghorn cockerels, which had no access to food; artificial cerebrospinal fluid (aCSF); MIN, minutes; LIN, linear contrast; QUAD, quadratic contrast; PSE, pooled standard error; There were no significant differences (P≤ 0.05).
Figure 4. Effect of intracerebroventricular injections of histamine (HA) on water consumption of broiler cockerels, which had no access to food; artificial cerebrospinal fluid (aCSF); MIN, minutes; LIN, linear contrast; QUAD, quadratic contrast; PSE, pooled standard error; There were no significant differences (P< 0.05).
Figure 5. Effect of intracerebroventricular injections of histamine (HA) body temperature of Single Comb White Leghorn cockerels; artificial cerebrospinal fluid (aCSF); LIN, linear contrast; QUAD, quadratic contrast; PSE, pooled standard error, +, significant difference (P≤ 0.05).
Figure 6. Effect of intracerebroventricular injections of histamine (HA) body temperature of broiler cockerels; artificial cerebrospinal fluid (aCSF); MIN, minutes; LIN, linear contrast; QUAD, quadratic contrast; PSE, pooled standard error, +, significant difference (P ≤ 0.05).
Figure 7. Effect of intracerebroventricular injections of chlorpheneramine maleate (CM) followed by histamine (HA) on food (A) and water consumption (B) of Single Comb White Leghorn cockerels; artificial cerebrospinal fluid (aCSF); + significant difference ($P \leq 0.05$); MIN, minutes; PSE, pooled standard error.
Figure 8. Effect of intracerebroventricular injections of chloropheneramine maleate (CM) followed by histamine (HA) on food (A) and water consumption (B) in broiler cockerels; artificial cerebrospinal fluid (aCSF); +, significant difference (P< 0.05); MIN, minutes; PSE, pooled standard error.
Figure 9. Effect of intracerebroventricular injections of cimetidine (CIM) followed by histamine (HA) on food (A) and water consumption (B) of Single Comb White Leghorn cockerels; artificial cerebrospinal fluid (aCSF); +, significant difference (P<0.05); MIN, minutes; PSE, pooled standard error.
Figure 10. Effect of intracerebroventricular injections of cimetidine (CIM) followed by histamine (HA) on food (A) and water consumption (B) of broiler cockerels; artificial cerebrospinal fluid (aCSF); +, significant difference (P<0.05); MIN, minutes; PSE, pooled standard error.
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CHAPTER TWO

THE INTERACTION OF CORTICOTROPIN RELEASING FACTOR AND HISTAMINE ON INGESTIVE BEHAVIOR IN THE DOMESTIC FOWL

ABSTRACT

Previous studies showed that intracerebroventricular injections of histamine (HA) and corticotropin releasing factor (CRF) decrease food intake. This experiment was conducted to determine if CRF elicits its effects on feeding through the release of HA. Single Comb White Leghorn (SCWL) and broiler cockerels were utilized for these experiments. Birds were stereotaxically implanted with a 23-gauge stainless steel cannula into the right lateral ventricle. Birds were infused with 0 or 20µg CRF, 0 or 100µg of a HA antagonist, or a combination of both. Chloropheneramine maleate (CM) was used as the H₁-antagonist and cimetidine (CIM) was utilized as the H₂-antagonist. Food and water consumption were monitored at 15-minute intervals through three hours postinjection. Food consumption was significantly decreased with the infusion of CRF (P< 0.05) in both SCWL and broiler cockerels. Water consumption was also decreased, however this effect was not observed until later time periods in both types of birds. When birds were pretreated with CM, the hypophagic effect of CRF was attenuated. Water consumption followed this pattern in broiler cockerels, however this was not seen in SCWL. When birds were pretreated with CM, the adipsic responses to CRF were not attenuated. Pretreatment with CIM attenuated the anorexigenic responses to CRF broiler cockerels. Although CRF decreased food intake in SCWL, the effect was not attenuated with the pretreatment of CIM. From these results, it appears that CRF may decrease food intake by causing the release of histamine within the central nervous system, a response opposite that of mammals.

Key Words: Chickens, Chloropheneramine maleate, Cimetidine, Corticotropin releasing factor (CRF), Food intake, Histamine, Water intake.
Corticotropin releasing factor (CRF) is a 41- amino acid peptide that has been isolated from extracts of ovine hypothalamus and characterized by Spiess et al. (1981). It is a putative neurotransmitter and/or neuromodulator within the central nervous system. It potently stimulates adrenocorticotropic hormone (ACTH) and β-endorphin secretion from the pituitary gland (Vale et al., 1981) in vivo and in vitro (Rivier et al., 1982). This control is exerted through projections from the paraventricular nucleus (PVN) to the median eminence that contains high concentrations of CRF (Petrusz et al., 1985). CRF is unevenly distributed within the brain, and is found in regions not associated with the control of pituitary function such as the nucleus accumbens, preoptic hypothalamus, the lateral hypothalamus (LA), parabrachial nucleus, and the dorsomotor nucleus of the vagus (Petrusz et al., 1985; Sawchenko and Swanson, 1985; Swanson et al., 1983).

When administered centrally, CRF produces potent behavioral effects including decreased food intake, increased movement and grooming in several species. This suggests that CRF may be a mediator in stress-related suppression of food intake (Morely and Levine, 1982). These effects are independent of its role in the hypothalamic-pituitary-adrenal axis (Morley and Levine, 1982).

CRF administration into the third ventricle of the rat produced an acute reduction of food intake of obese rats (Arase et al., 1989). CRF also decreased food and water intake by 30-50% when administered intracerebroventricularly (ICV), but not systematically to sheep (Ruckebush and Malbert, 1986). CRF acts within the central nervous system of chickens to decrease food intake, although it was reported not to have any affect on water intake (Denbow, 1999). When various pharmacological agents including insulin, norepinephrine, muscimol, and dynorphin known to produce a marked increase in food intake were administered icv, their action was antagonized by CRF (Levine et al. 1983). This suggests that CRF may represent an important agent in stress-induced anorexia.

Histamine (HA) is a putative neurotransmitter. It is synthesized from the amino acid precursor histidine which is decarboxylated to HA in a restricted area located in the tuberomammilary nucleus of the posterior hypothalamus (Leurs and Timmerman, 1997). Peripherally synthesized HA does not cross the blood brain barrier. Histamine can act on H₁
and H-2 receptors causing changes in the release of corticotropin releasing factor (CRF), thus producing responses in discrete nuclei related to food intake, such as the ventromedial nucleus (VMH) and paraventricular nucleus (PVN) (Ookuma et al., 1988, 1993; Mercer et al., 1990).

ICV injections of HA and its amino acid precursor histidine depress food intake in rats (Fukagawa et al., 1989). The sites of histaminergic modulation of food intake, were identified when H-1-receptor antagonists were infused into rat hypothalamus, ventromedial hypothalamus (VMH), the lateral hypothalamus (LHA), the paraventricular nucleus (PVN), the dorsomedial hypothalamus (DMH), or the preoptic anterior hypothalamus (POAH) (Ookuma et al. 1988, 1993; Sakata et al. 1988a,b). Feeding was induced by bilateral infusion into the VMH and PVN. These findings suggested that H-1-receptor agonists infused bilaterally into the VMH and PVN were involved in histaminergic suppression of food intake with diurnal variations.

Injection of either histamine, H-1, or H-2 agonists, causes the release of corticotropin releasing factor (CRF), adrenocorticotropic hormone (ACTH), and corticosterone (Mercer et al., 1990). Alterations in brain histamine concentration are also associated with changes in feeding, drinking, neuroendocrine secretion, the sleep-wakefulness cycle, thermoregulation, and pain threshold (Doi et al., 1994). Ookuma et al. (1997) reported that injections of HA into the POAH of the rat lowered body temperature. This effect was blocked by H-1-antagonists.

Since CRF and histamine have both been shown to decrease food intake when injected into the hypothalamus, the purpose of this study was to determine if CRF decreases food intake by altering the release of histamine.
MATERIALS AND METHODS

Animals. Broiler and Single Comb White Leghorn (SWCL) cockerels were reared in heated batteries with continuous lighting until 4 and 7 weeks of age, respectively. Birds were provided a mash diet (20% crude protein, 3.0% crude fat, and 2864 kcal/kg of metabolizable energy) and water for ad libitum consumption. At approximately 3 and 6 weeks of age, the birds were transferred to individual cages measuring 17.6 x 26.4 x 17.6 cm. Each cage was supplied with individual feeders and waterers with continuous lighting.

Surgical preparation. At 4 and 7 weeks of age, broilers and SCWL were anesthetized with sodium pentobarbital (25 mg/kg body weight, intravenously; iv) and a 23-gauge thin-walled stainless steel guide cannula was stereotaxically implanted into the right lateral cerebral ventricle as described by Denbow et al. (1981). The cannula was secured with 3 stainless steel screws placed in the calvaria surrounding each guide cannula and acrylic dental cement (Cranioplast Plastics Product Company, Roanoke, VA) was applied to the screws and guide cannula. Placement of the cannula into the ventricle was verified the presence of cerebrospinal fluid. Birds were allowed a minimum of 2 days recovery prior to injection.

Solutions. Artificial cerebrospinal fluid (aCSF; Anderson and Heisey, 1972) consisted of (NaCl .754g; KCl .028g; CaCl₂ .018; MgCl₂ 6H₂O .024g; NaHCO₃ .194g; NaHPO₄ .02; Ascorbic acid .1 g/100ml) which was filtered through a .22µm filter (Gelman Instrument Co., Ann Arbor, MI) prior to any injections.

Experiment 1. This experiment evaluated the effect of ICV administered corticotropin releasing factor (CRF) (Sigma), and a histamine H₁-receptor antagonist chlorpheneramine maleate (Sigma). Eight Leghorn cockerels were used in a replicated Latin Square design in which birds and days were the blocking factors. All solutions were prepared in aCSF, which was also used as a control. Each bird received two injections. The first injection consisted of either 0 or 100 µg chlorpheneramine maleate in a volume of 5 µl. The second injection consisted of either 0 or 20 µg CRF in a volume of 10 µl. Injections were made using a 27-gauge stainless steel injection cannula connected to a 10 µl Hamilton syringe connected to a 60 cm length of PE-20 tubing (Clay Adams). Food and water intake was monitored at 15-minute intervals through three hours postinjection.
Experiment 2. The experiment was similar to Experiment 1 except broilers were used instead of SCWL.

Experiment 3. This experiment evaluated the effect of ICV administered corticotropin releasing factor (CRF) (Sigma), and a histamine H$_2$-receptor antagonist cimetidine (Sigma). Eight Leghorn cockerels were used in a replicated Latin Square design in which birds and days were the blocking factors. All solutions were prepared in aCSF, which was also used as a control. Each bird received two injections. The first injection consisted of either 0 or 100µg cimetidine in a volume of 5µl. The second injection consisted of either 0 or 20µg CRF in a volume of 10µl. Injections were made using a 27-gauge stainless steel injection cannula connected to a 10µl Hamilton syringe connected to a 60cm length of PE-20 tubing (Clay Adams). Food and water intake was monitored at 15-minute intervals for three hours postinjection.

Experiment 4. The experiment was similar to Experiment 3 except broilers were used instead of SCWL.

Experiment 5. This experiment evaluated the effect of ICV administered corticotropin releasing factor (CRF) (Sigma), and a histamine H$_3$-receptor antagonist bombesin (Sigma). Eight Leghorn cockerels were used in a replicated Latin Square design in which birds and days were the blocking factors. All solutions were prepared in aCSF, which was also used as a control. Each bird received two injections. The first injection consisted of either 0 or 100µg bombesin in a volume of 5µl. The second injection consisted of either 0 or 20µg CRF in a volume of 10µl. Injections were made using a 27-gauge stainless steel injection cannula connected to a 10µl Hamilton syringe connected to a 60cm length of PE-20 tubing (Clay Adams). Food and water intake was monitored at 15-minute intervals for three hours postinjection.

Experiment 6. The experiment was similar to Experiment 5 except broilers were used instead of SCWL.

Analysis. Cumulative food and water intake was analyzed using an analysis of variance at each time period. Duncan’s multiple range test and non-orthogonal contrasts were used for obtaining all pairwise comparisons among sample means at each time period. Significance was set to $P \leq 0.05$. 
RESULTS AND DISCUSSION

The food and water intake responses to intracerebroventricular (ICV) injections of corticotropin releasing factor (CRF) and H₁-receptor antagonist chlorphenemeramine maleate (CM) for Single Comb White Leghorn (SCWL) and broiler cockerels are summarized in Figures 1 and 2, respectively. Food consumption was significantly, \( P < 0.05 \) decreased by the infusion of CRF in both SCWL and broiler cockerels. Water consumption was similarly decreased. Pretreatment with CM attenuated the CRF-induced hypophagia in broilers and SCWL. CM also attenuated the adipsic response of CRF in broilers, however, it did not attenuate it in SCWL. There was a decrease in water intake when SCWL were pretreated with CM.

The food and water intake responses to ICV injections of CRF and H₂-receptor antagonist, cimetidine (CIM) for SCWL and broiler cockerels are summarized in Figures 3 and 4, respectively. The anorexic effect of CRF was attenuated by pretreatment with CIM in SCWL and broiler cockerels. Water consumption showed a similar response to food intake.

Considerable evidence exists that suggest CRF neurons in the PVN mediate a suppression of food intake. When CRF is applied ICV, it has an anorexic effect in the rat (Arase et al., 1989) and in the chicken (Denbow, 1999). CRF levels are also elevated in the cerebrospinal fluid of patients with anorexia nervosa (Hotta et al. 1986) as well as patients inflicted with depression (Nemeroff et al. 1984).

Histamine mediates a suppression of food intake in several species. H₁ receptor antagonists induced feeding in rats (Sakata et al., 1988b). Mercer et al. (1990) postulated a possible pathway for the involvement of HA and CRF in the regulation of food intake. A dietary deficiency in protein or indispensable amino acids leads to a decrease in the hepatic enzyme histidase secondary to a combination of decreased rates of protein synthesis and increased protein metabolism. Histidine then crosses the blood brain barrier leading to an increased synthesis in the hypothalamus. This increase activates histaminergic systems producing an effect involving increased activity of CRF, thus producing a depressant effect on food intake (Mercer et al., 1990).

It is well established that central administration of CRF reduces food intake in rats, chickens and sheep. The present study supports the hypothesis that CRF may be a potent suppressor of ingestive behavior in chickens. However, the postulate that HA causes the release
of CRF does not seem to apply to chickens. From these results, it appears that CRF may decrease food intake by causing the release of HA within the CNS, a result opposite that of mammals.
Figure 1. Effect of intracerebroventricular injections of chlorpheneramine maleate (CM) followed by corticotropin releasing factor (CRF) on food (A) and water consumption (B) of Single Comb White Leghorn cockerels; artificial cerebrospinal fluid (aCSF), +, significant difference (P<0.05); MIN, minutes; PSE, pooled standard error.
Figure 2. Effect of intracerebroventricular injections of chlorpheneramine maleate (CM) followed by corticotropin releasing factor (CRF) on food (A) and water consumption (B) of broiler cockerels; artificial cerebrospinal fluid (aCSF), +, significant difference (P<0.05); MIN, minutes; PSE, pooled standard error.
Figure 3. Effect of intracerebroventricular injections of cimetidine (CIM) followed by corticotropin releasing factor (CRF) on food (A) and water consumption (B) of Single Comb White Leghorn (SCWL) cockerels; artificial cerebrospinal fluid (aCSF); +, significant difference (P<0.05); MIN, minutes; TSE, treatment standard error.
Figure 4. Effect of intracerebroventricular injections of cimetidine (CIM) followed by corticotropin releasing factor (CRF) on food (A) and water consumption (B) of broiler cockerels; artificial cerebrospinal fluid (aCSF); +, significant difference (P≤0.05); MIN, minutes; PSE, pooled standard error.
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CHAPTER THREE

THE INTERACTION OF BOMBESIN AND CORTICOTROPIN-RELEASING HORMONE ON INGESTIVE BEHAVIOR IN THE DOMESTIC FOWL

ABSTRACT

An experiment was conducted to investigate if bombesin (BM) elicits its effects on feeding through the release of corticotropin releasing factor (CRF). Single Comb White Leghorn (SCWL) and broiler cockerels were stereotaxically implanted with a 23-gauge stainless steel cannula into the right lateral ventricle. Birds were infused with 0 or 0.5 µg BM, 5 µg αCRF (a CRF antagonist), or a combination of both. Food and water consumption were monitored at 15-minute intervals through three hours postinjection. Food and water consumption were both significantly decreased (P ≤ 0.05) by BM in both SCWL and broilers. In SCWL, αCRF had no affect on food intake by itself, but attenuated the effects of BM. In broilers, αCRF caused a slight, but significant decrease, in food intake, but also attenuated the effects of BM. Water consumption was not affected by αCRF in either broilers or SCWL. It is concluded that BM may mediate its central effects on food intake in chickens through the release of CRF.

Key Words: αCRF, bombesin, chickens, CRF antagonist, food intake, water intake,
INTRODUCTION

Bombesin (BM) is a tetradecapeptide derived from amphibians (Chronwall et al., 1985; Moody et al., 1986). It is located in discrete forebrain and hindbrain sites such as the paraventricular nucleus (PVN), the nucleus tractus solitarius (NTS), interpeduncular nucleus, central grey, dorsolateral tegmental nucleus, dorsoparabrachial nucleus, nucleus of the solitary tract and trigeminal complex (Panula et al., 1982). The densest of these BM fibers are found in the hypothalamus (Merali and Banks, 1994; Plamondon and Merali, 1997b). Intense immunoreactivity was also found in superficial layers of the posterior horn of the spinal cord (Panula et al., 1982).

As with several other peptides, BM is hypothesized to have a role in food intake regulation. However, the mechanism of action remains unclear. Administration of BM suppresses food intake in diverse species such as rats (Flynn, 1989) and turkeys (Denbow, 1988). Central or systemic administration of BM has a number of other biologically significant actions, including elevation of blood glucose (Brown et al., 1979; Plamondon and Merali, 1993) and elicitation of grooming (Gmerek and Cowan, 1983; Pert et al., 1980) and locomotor activity (Merali et al. 1994). In rats, BM appears to decrease food intake acting in the brain; but not in the fourth ventricle of rats (Flynn, 1989). Denbow (1988) found that intracerebroventricular (ICV) as well as peripheral injections of BM decreased food and water intake in a dose-dependent manner in turkeys. Interestingly, BM decreases water intake in rats when food was available, however, when food was removed, there was no effect on food intake (Flynn, 1989).

Systemically administered BM produces a satiety-like effect in several species including lean and genetically obese mice (McLaughlin and Baile, 1981; Taylor et al. 1985), hamsters (Miceli and Malsbury, 1985), and turkeys (Denbow, 1988). In addition, iv infusions of BM caused a dose-related inhibition of food intake of test meals in baboons (Woods et al., 1983). Stuckey et al. (1985) found that the neurally disconnecting the gut from the brain completely abolished feeding suppressant effects of systemically administered BM. This suggests that there is a peripheral site of action for systemically administered BM. Merali et al. (1993) suggested that the effect of systemically administered BM is neurally communicated to the brain where BM receptors in the caudal brain stem sites are involved in the suppression of food intake.
Corticotropin releasing factor (CRF) is a 41 amino acid peptide, synthesized from a precursor containing 196 amino acids. It has been reported to be a putative neurotransmitter and/or neuromodulator within the central nervous system (CNS) (Emoto et al., 1993). CRF has been characterized from extracts of ovine hypothalamus (Vale et al., 1981). CRF is a potent stimulator of pituitary secretion of adrenocorticotropic hormone (ACTH) and β-endorphin both in vivo and in vitro (Rivier et al., 1982). This control is exerted through projections from the PVN to the median eminence that contains high concentrations of CRF (Swanson et al., 1983; Petrusz et al., 1985).

Central administration of CRF decreases food intake in rats, (Britton et al. 1982; Morley and Levine, 1982) and chickens (Denbow et al., 1999, in press). CRF has been reported to suppress feeding when microinjected into the PVN, but not LHA or VMN, globus pallidus or corpus striatum (Morley and Levine, 1982).

Like that of BM, central administration of CRF decreases food intake. Panula et al. (1982) observed that the autonomic and behavioral syndromes that are elicited by CRF strongly resemble that of BM and that there may be some anatomical overlap of these two peptides. Familiari et al. (1988) demonstrated that BM was consistently potentiating the release of ACTH in response to CRF pulses which suggested that it potentiates CRF stimulated ACTH release. The potentiation of CRF by BM was glucocorticoid dependent.

This was supported by the observation of the interaction between these two peptides demonstrating that many of the autonomic and endocrine effects of exogenous BM are blocked by central pretreatment with the CRF antagonist, αCRF (Merali et al., 1994). Plamondon and Merali (1997a) also demonstrated that CRF mediated the anorexigenic effects of BM. Central administration of two CRF antagonists blocked the behavioral and feeding suppressant actions of BM. However, administration of αCRF alone failed to affect food intake. Blockade of central CRF receptors prevents BM’s suppressant action on feeding suggesting that BM may elicit its effects on feeding and related behaviors through the release of central CRF pathways (Plamondon and Merali, 1997b; Garrida et al. 1998). The precise receptor-based mechanism by which CRF and BM may interact however, still remains unknown.

There have been several studies examining the effects of BM and CRF, on feed and water intake using rodents. However, very little work has been done to study the effects of these
compounds in the avian species. Therefore, this study was conducted to determine if BM elicits its effects on feeding through the release of in avian species.
MATERIALS AND METHODS

Animals. Broiler and Single Comb White Leghorn (SWCL) cockerels were reared in heated batteries with continuous lighting until 4 and 7 weeks of age, respectively. Birds were provided a mash diet (20% crude protein, 3.0% crude fat, and 2864 kcal/kg of metabolizable energy) and water for ad libitum consumption. At approximately 3 and 6 weeks of age, the birds were transferred to individual cages measuring 17.6 x 26.4 x 17.6cm. Each cage was supplied with individual feeders and waterers, and continuous lighting was provided.

Surgical preparation. At 4 and 7 weeks of age broilers and SCWL were anesthetized with sodium pentobarbital (25mg/kg body weight, iv), and a 23-gauge thin-walled stainless steel guide cannula was stereotaxically implanted into the right lateral cerebral ventricle as previously described (Denbow et al., 1981). The cannula was secured with 3 stainless steel screws placed in the calvaria surrounding each guide cannula and acrylic dental cement (Cranioplast Plastics Product Company, Roanoke, VA) was applied to the screws and guide cannula. Placement of the cannula into the ventricle was verified by the presence of cerebrospinal fluid in the cannula. Birds were allowed a minimum of 2 days recovery prior to injection.

Solutions. Artificial cerebrospinal fluid (aCSF; Anderson and Heisey, 1972) consisted of NaCl .754g; KCl .028g; CaCl2 .018; MgCl 6H2O .024g; NaHCO3 .194g; NaHPO4 .02; Ascorbic acid .1g/100ml which was filtered through a .22µm filter (Gelman Instrument Co., Ann Arbor, MI) prior to any injections.

Experiment 1. This experiment evaluated the effect of ICV administered alpha corticotropin releasing factor (αCRF (9-41)) (Sigma), and bombesin (Sigma). Eight Leghorn cockerels were used in a replicated Latin Square design in which birds and days were the blocking factors. All solutions were prepared in aCSF, which was also used as a control. Each bird received two injections. The first injection consisted of either 0 or 5µg bombesin in a volume of 5µl. In a factorial arrangement, the second injection consisted of either 0 or 0.5µg αCRF in a volume of 5µl. Injections were made using a 27-gauge stainless steel injection cannula via a 10µl Hamilton syringe connected to a 60cm length of PE-20 tubing (Clay Adams). Food and water intake was monitored at 15-minute intervals for three hours postinjection.
**Experiment 2.** The experiment was similar to Experiment 1 except broilers were used instead of SCWL.

*Analysis.* Cumulative food and water intake was analyzed using analysis of variance at each time period. Duncan’s multiple range test and non-orthogonal contrasts were used for obtaining all pairwise comparisons among sample means at each time period. Significant differences imply to $P \leq 0.05$. 
RESULTS AND DISCUSSION

The food and water responses of ICV injections of BM and αCRF for SCWL and broiler cockerels are summarized in Figures 1 and 2, respectively. ICV injections of αCRF did not elicit any effects on feeding or water consumption when infused into the right lateral ventricle of SCWL. However, when αCRF was administered ICV to broiler cockerels, there was a decrease in food and water consumption compared to the control. Bombesin significantly decreased food, but not water consumption, in both SCWL and broiler cockerels. The decrease in food intake caused by ICV injection of BM was attenuated by the pretreatment with αCRF in both SCWL and broilers.

Pretreatment of birds with αCRF followed by BM and the diminished anorexigenic effects of BM implies that there may be some overlap in the action of these two neurotransmitters. When administered alone, αCRF failed to affect food intake, suggesting that the blockade of BM-induced anorexia was not attributed to intrinsic properties of these drugs. Plamondon and Merali (1997a) observed that BM did interact with αCRF. However, it did not interact with oxytocin nor the CRF antagonist. These results are consistent with the notion that BM may mediate its behavioral effects partly through the activation of the central CRF system.

Both BM (Denbow, 1988; Flynn, 1989) and CRF (Britton et al., 1982; Denbow et al., 1999, in press) have been shown to induce satiety in a variety of species when infused centrally. Although the mechanism in which these two peptides exert their effects on feeding is unclear, there is evidence of anatomical overlap between the two neuronal systems. There have been studies suggesting that BM may mediate its satiety effects through the histaminergic system (Merali and Banks, 1994). Similarly, BM has been suggested to exert its effects on feeding through the release of CRF (Merali et al., 1994; Plamondon and Merali, 1997b). Plamondon and Merali (1997b) reported results similar to those of the present study. Central pretreatment with CRF antagonists blocked the effects of centrally administered BM. Their results provided evidence that BM mediates its anorexigenic effects through the release of CRF. However, when CRF antagonists were administered alone, they had no effect on food intake, similar to present findings. This is consistent with work done by Merali et al. (1994) that pretreatment with αCRF blocked endocrine and autonomic effects of centrally administered BM.
Although discrete brain sites have not been identified, the PVN may play a role in food intake since meal related changes in the release of BM have previously been reported (Merali et al., 1994). Therefore it may be possible that an increased BM release may stimulate the release of CRF from neurons in the PVN.
Figure 1. Effect of intracerebroventricular injections of bombesin (BM) and alpha CRF (αCRF) on food (A) and water (B) consumption of Single Comb White Leghorn cockerels; artificial cerebrospinal fluid (aCSF); MIN, minutes; PSE, pooled standard error.
Figure 2. Effect of intracerebroventricular injections of bombesin (BM) and alpha CRF (αCRF) on food (A) and water (B) consumption of broiler cockerels; artificial cerebrospinal fluid (aCSF). MIN, minutes; PSE, pooled standard error.
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SUMMARY

These sets of experiments were designed to demonstrate the role of histamine and its interaction with bombesin and corticotropin releasing factor (CRF). Histamine was shown to decrease food and water intake in both Single Comb White Leghorn and broiler cockerels. CRF also induced hypothermia in SCWL. In contrast, ICV injections of CRF caused hyperthermia in broilers. The anorexigenic effects of histamine were blocked with the pretreatment of H$_1$-receptor antagonist chloropheneramine maleate (CM) and H$_2$-receptor antagonist cimetidine (CIM) in both broilers and SCWL. From these results, it was concluded that histamine decreases food intake acting through both H$_1$ and H$_2$ receptors.

The next pair of experiments examined the interaction of histamine and CRF. Previous studies reported that hypothalamic histamine and H$_1$ and H$_2$ agonists cause the release of CRF. However when birds were pretreated with the histamine antagonists CM and CIM, the hypophagic effects of CRF were attenuated. From these results, it was concluded that CRF may mediate its effects on feeding through the release of HA.

The last experiments examined the interaction of bombesin (BM) and CRF. Knowing that CRF and BM decreased food intake, and that there were some anatomical and endocrine similarities between the two, experiments were conducted to examine if BM exerted its effects through CRF. Central effects of BM on food intake were attenuated with the pretreatment of $\alpha$CRF. From these results, it was concluded that BM mediates its effects on feeding through CRF release.

Together all of these experiments were designed to understand how histamine influences food intake in chickens and how histamine interacts with other neurotransmitters. It is now understood that histamine acts upon H$_1$ and H$_2$ receptors to influence food intake in chickens. The conclusion to these experiments is as follows: One component of the neuroregulation of food intake in chickens is that BM mediates its effects on feeding through the release of CRF, and CRF may mediate its effects on feeding through the release of HA in chickens.