A Review of Neuropeptide and Neuroendocrine Dysregulation in Anorexia and Bulimia Nervosa

Ursula F. Bailer and Walter H. Kaye*

University of Pittsburgh Medical Center, Anorexia and Bulimia Nervosa Research Module, Western Psychiatric Institute & Clinic, 3811 O'Hara Street, 600 Iroquois Building, Pittsburgh, PA 15213, USA

Abstract: Neuropeptides play an important role in the regulation of feeding behavior and obesity. The mechanisms for controlling food intake involve a complicated interplay between peripheral systems (including gustatory stimulation, gastrointestinal peptide secretion, and vagal afferent nerve responses) and central nervous system (CNS) neuropeptides and/or monoamines. These neuronal systems include neuropeptides (CRH, opioids, neuropeptide-Y (NPY) and peptide YY (PYY), vasopressin and oxytocin, CCK, and leptin) and monamines (serotonin, dopamine, norepinephrine). In addition to regulating eating behavior, a number of CNS neuropeptides participate in the regulation of neuroendocrine pathways. Thus, clinical studies have evaluated the possibility that CNS neuropeptide alterations may contribute to dysregulated secretion of the gonadal hormones, cortisol, thyroid hormones and growth hormone in the eating disorders. Most of the neuroendocrine and neuropeptide alterations apparent during symptomatic episodes of AN and BN tend to normalize after recovery. This observation suggests that most of the disturbances are consequences rather than causes of malnutrition, weight loss and/or altered meal patterns. Still, an understanding of these neuropeptide disturbances may shed light on why many people with AN or BN cannot easily "reverse" their illness and even after weight gain and normalized eating patterns, many individuals who have recovered from AN or BN have physiological, behavioral and psychological symptoms that persist for extended periods of time.

Key Words: eating disorders, neuropeptides, neuroendocrinology, anorexia nervosa, bulimia nervosa.

NEUROPEPTIDES

The past decade has witnessed accelerating basic research on the role of neuropeptides in the regulation of feeding behavior and obesity. The mechanisms for controlling food intake involve a complicated interplay between peripheral systems (including gustatory stimulation, gastrointestinal peptide secretion, and vagal afferent nerve responses) and central nervous system (CNS) neuropeptides and/or monoamines. Thus, studies in animals show that neuropeptides, such as cholecystokinin, the endogenous opioids (such as beta-endorphin), and neuropeptide-Y, regulate the rate, duration, and size of meals, as well as macronutrient selection [1,2]. In addition to regulating eating behavior, a number of CNS neuropeptides participate in the regulation of neuroendocrine pathways. Thus, clinical studies have evaluated the possibility that CNS neuropeptide alterations may contribute to dysregulated secretion of the gonadal hormones, cortisol, thyroid hormones and growth hormone in the eating disorders [3,4].

While there are relatively few studies to date, most of the neuroendocrine and neuropeptide alterations apparent during symptomatic episodes of AN and BN tend to normalize after recovery. This observation suggests that most of the disturbances are consequences rather than causes of malnutrition, weight loss and/or altered meal patterns. Still, an understanding of these neuropeptide disturbances may shed light on why many people with AN or BN cannot easily "reverse" their illness. In AN, malnutrition may contribute to a downward spiral sustaining and perpetuating the desire for more weight loss and dieting. Symptoms such as increased satiety, obsessions and dysphoric mood, may be exaggerated by these neuropeptide alterations and thus contribute to this downward spiral. Additionally, mutual interactions between neuropeptide, neuroendocrine and neurotransmitter pathways may contribute to the constellation of psychiatric comorbidity often observed in these disorders. Even after weight gain and normalized eating patterns, many individuals who have recovered from AN or BN have physiological, behavioral and psychological symptoms that persist for extended periods of time. Menstrual cycle dysregulation, for example, may persist for some months after weight restoration. The following sections provide a brief overview of studies of neuropeptides in AN and BN.

OPIOID PEPTIDES

Studies in laboratory animals raise the possibility that altered endogenous opioid activity might contribute to pathological feeding behavior in eating disorders since opioid agonists generally increase, and opioid antagonists decrease, food intake [5]. State-related reductions in concentrations of CSF beta-endorphin and related opiate

*Address correspondence to the author at the University of Pittsburgh Medical Center, Anorexia and Bulimia Nervosa Research Module, Western Psychiatric Institute & Clinic, 3811 O'Hara Street, 600 Iroquois Building, Pittsburgh, PA 15213, USA; Tel: (412) 647-9845; Fax: (412) 647-9740; E-mail: kayewh@msx.upmc.edu

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concentrations have been found in both underweight AN and ill BN subjects [6-8]. In contrast, using the T-lymphocyte as a model system, Brambilla *et al.* [9] found elevated beta-endorphin levels in AN, although the levels were normal in BN [9]. If beta-endorphin activity is a facilitator of feeding behavior, then reduced CSF concentrations could reflect decreased central activity of this system, which then maintains or facilitates inhibition of feeding behavior in the eating disorders.

A disturbance in CNS opioid function may also contribute to the neuroendocrine abnormalities in anorexia and bulimia nervosa (e.g. disturbances in HPA and pituitary-gonadal axis function) [10,11]. Brain opioid pathways inhibit ACTH and cortisol release in humans, and they suppress pulsatile gonadotropin secretion in rats and in sexually mature humans. Underweight anorexics frequently have a blunted response of LH secretion to opiate antagonists [12] and weight restoration tends to normalize this response. The failure of opioid antagonists to increase LH secretion in underweight anorexics suggests that another neurotransmitter system (or systems) may be responsible for this neuroendocrine disturbance.

Corticotropin Releasing Hormone (CRH.)

When underweight, patients with AN have increased plasma cortisol secretion that is thought to be at least in part a consequence of hypersecretion of endogenous CRH [13-16]. In that the plasma and cerebrospinal fluid (CSF) measures return toward normal, it appears likely that activation of the HPA axis is precipitated by weight loss. The observation of increased CRH activity is of great theoretical interest in AN since intracerebroventricular CRH administration in experimental animals produces many of the physiologic and behavioral changes associated with AN, including markedly decreased eating behavior [17], hypothalamic hypogonadism [18], decreased sexual activity [19], and hyperactivity [20].

Vasopressin and Oxytocin

In addition to the effects of vasopressin on HPA axis regulation and free-water clearance by the kidney and the effects of oxytocin during the puerperium, these structurally related neuropeptides are distributed throughout the CNS and function as long-acting neuromodulators of complex behaviors. The effects of vasopressin appear to be reciprocal to those of oxytocin: Central administration of vasopressin to rats enhances memory consolidation and retrieval, whereas administration of oxytocin disrupts memory [21].

In addition to abnormally high CSF vasopressin concentrations and impaired osmoregulation of plasma vasopressin [22], anorexia nervosa patients have reduced CSF oxytocin concentrations and impaired plasma oxytocin responses to stimulation [23]. Underweight anorexics also have an impaired plasma oxytocin response to challenging stimuli [24]. These abnormalities tend to normalize after weight restoration suggesting they are secondary to malnutrition or abnormal fluid balance, or both. In underweight anorexics, low CNS oxytocin might interact with high CNS vasopressin to enhance the retention of cognitive distortions of the aversive consequences of eating, thereby reinforcing these patients' perseverative preoccupation with the adverse consequences of food intake.

Patients with normal-weight bulimia were found to have elevated CSF vasopressin concentrations but normal CSF oxytocin both on admission and after 1 month of nutritional stabilization and abstinence from bingeing and purging. In these patients, as well, CNS vasopressin might contribute to their obsessional preoccupation with the aversive consequences of weight gain [25].

Neuropeptide-Y (NPY) and Peptide YY (PYY)

These peptides are of considerable theoretical interest since they are among the most potent endogenous stimulants of feeding behavior within the CNS [2,5,26]. PYY is more potent than NPY in stimulating food intake; both are selective for carbohydrate rich foods. In vivo measurements have shown that NPY released from the paraventricular hypothalamus increases during hunger and falls during meals [26]. The appetite-stimulating effect of NPY is inhibited by corticotropin-releasing hormone (CRH) [27,28]. There is increasing evidence that a dynamic equilibrium exists between NPY and CRH neuronal activity, and this system is important for the regulation of food intake [29]. Underweight individuals with AN have been shown to have elevations of CSF NPY, but normal PYY [30]. Clearly, elevated NPY does not result in increased feeding in individuals underweight with AN; however, the possibility that increased NPY activity underlies the obsessive and paradoxical interest in dietary intake and food preparation is a hypothesis worth exploring. On the other hand, CSF levels of NPY and PYY have been reported to be normal in women with BN when measured while subjects were acutely ill. Although levels of PYY increased above normal when subjects were re-assessed after one month of abstinence from bingeing and vomiting, levels of the peptides were similar to control values in long-term recovered individuals [31]. More recently, it has been reported that the plasma concentration of NPY was lower in AN patients than in controls, while BN patients had elevated NPY levels [32]. Additional studies will be needed to assess the potential behavioral correlates of these findings.

Cholecystokinin (CCK)

CCK is a peptide secreted by the gastrointestinal system in response to food intake. Release of CCK is thought to be one means of transmitting satiety signals to the brain by way of vagal afferents [33]. In parallel to its role in satiety in rodents, exogenously administered CCK reduces food intake in humans. The preponderance of data suggests that patients with BN, in comparison to controls, have diminished release of CCK following ingestion of a standardized test-meal [34-37]. Measurements of basal CCK values in blood lymphocytes and in CSF also appear to be decreased in patients with BN [9,38]. It has been suggested that the diminished CCK response to a meal may play a role in

diminished post-ingestive satiety observed in BN. The CCK response in BN patients was found to return toward normal following treatment [35].

Studies of CCK in AN have yielded less consistent findings. Some studies have found elevations in basal levels of plasma CCK [36,39], as well as increased peptide release following a test-meal [36,40]. One study found that blunting of CCK response to an oral glucose load normalized in AN patients after partial restoration of body weight [39]. Other studies have found that measures of CCK function in AN were similar to or lower than control values [37,41-43]. Further studies are needed to evaluate the relationship between altered CCK regulation and other indices of abnormal gastric function in symptomatic BN and AN patients [44].

Leptin

Leptin, the protein product of the *ob* gene, is secreted predominantly by adipose tissue cells, and acts in the CNS to decrease food intake, thus regulating body fat stores. In rodent models, defects in the leptin coding sequence resulting in leptin deficiency or defects in leptin receptor function are associated with obesity. There is evidence that the appetite-suppressive effect of leptin is mediated by an inhibition of the NPY secretion. [45,46]. However, NPY does not seem to be the only mediator, since mice deficient in NPY also responded to exogenous leptin administration [47]. In humans, serum and CSF concentrations of leptin are positively correlated with fat mass in individuals in across a broad range of body weight, including obesity [46,48]. Thus, obesity in humans is not thought to be a result of leptin deficiency per se, although rare genetic deficiencies in leptin production have been associated with familial obesity [49].

Underweight patients with AN have consistently been found to have significantly reduced serum leptin concentrations in comparison to normal weight controls. [41,50-53]. Based on studies in laboratory animals, it has been suggested that low leptin levels may contribute to amenorrhea and other hormonal changes in the disorder [53]. Although the reduction in fasting serum leptin levels in AN is correlated with reduction in body mass index, there has been some discussion of the possibility that leptin levels in AN patients may be higher than expected based on the extent of weight loss [54-55]. Mantzoros *et al.* [53] reported an elevated CSF to serum leptin ratio in AN compared to controls, suggesting that the proportional decrease in leptin levels with weight loss is greater in serum than in CSF. A longitudinal investigation during refeeding in AN patients has shown that CSF leptin concentrations reach normal values before full weight restoration, possibly as a consequence of the relatively rapid and disproportionate accumulation of fat during refeeding [53]. This finding led the authors to suggest that premature normalization of leptin concentration might contribute to difficulty in achieving and sustaining a normal weight in AN. Plasma and CSF leptin levels appear to be similar to control values in long-term recovered AN subjects [31].

Recent studies indicate that patients with BN, in comparison to carefully matched controls, have significantly decreased leptin concentrations in serum samples obtained after overnight fast [32,54,56-58]. Initial findings in individuals who have achieved sustained recovery from BN, when compared to controls with closely matched percent body fat, suggest that serum leptin levels remain decreased. This finding may be related to evidence for a persistent decrease in activity in the hypothalamic-pituitary-thyroid axis in long-term recovered BN individuals. These alterations could be associated with decreased metabolic rate and a tendency toward weight gain, contributing to the preoccupation with body weight characteristic of BN.

Knowledge about leptin-binding proteins and sensitivity is still very limited. It has been shown that significantly higher proportions of total leptin circulate in bound form in lean subjects compared with those in obese subjects [59]. It is feasible that leptin-binding proteins could themselves be involved in the regulation of eating behaviour.

Ghrelin

Ghrelin was originally discovered in the rat and the human stomach, and stimulates growth hormone secretion in rodents. This petide that antagonizes leptin action has a role in the regulation of feeding behavior and energy metabolism in the central nervous system [60]. Ghrelin-producing neurons are located in the hypothalamus, whereas, ghrelin receptors are expressed in various regions of the brain. Intracerebroventricular injections of ghrelin strongly stimulated feeding in rats and increased body weight gain. In addition it has been reported that fasting plasma ghrelin concentrations in humans are negatively correlated with BMI [61,62], percentage body fat and fasting leptin and insulin concentrations [63], which play an important role in the pathophysiology of anorexia nervosa [64]. In the latter study it could be shown, that ghrelin was elevated in anorexia nervosa patients and returned to normal levels after weight recovery. A possible existence of ghrelin resistance in cachectic states as caused by eating disorders could be suggested. Fasting plasma ghrelin concentrations in patients with bulimia nervosa were significantly higher than those in controls [62], although the BMIs between bulimics and controls were not significantly different, suggesting that not only BMI had an influence on circulating ghrelin level in BN patients, but also abnormal eating behavior with bingeing and purging.

Gastrin releasing peptide

Human gastring releasing peptide (GRP) is a 27 amino acid peptide that shares a similar decapeptide with bombesin (BBS) [65]. Peripheral and central administration of GRP attenuates food intake in mammals and humans [66,67]. In the central nervous system (CNS), distinct BBS-like receptor subtypes have been identified in brain tissue such as the bed nucleus of the stria terminalis, the olfactory tubercle, the putamen and neocortex, with a neuromedin B and a GRP preferring subtype [68,69]. Both subtypes have have been implicated in the modulation of BBS-like peptide induced

food suppression [70]. CSF GRP was significantly lower in recovered bulimic patients (> 1 year, normal weight, and regular menstrual cycles, no bingeing or purging) compared to normal controls and recovered anorectic patients [71]. Lower CSF GRP in this group could be a trait related disturbance that might add to hyperphagic behavior, and thus to the pathophysiology of this illness.

NEUROENDOCRINOLOGY

Abnormal hormone profiles and responses to challenge are closely related to the "starvation" status of anorexia nervosa and and bulimia nervosa patients. Hormone abnormalities may also be present, but to a lesser extent, in normal-weight women with bulimia nervosa. The presence of starvation in anorexia nervosa is evident from the weight loss, but it may not be recognized in normal-weight bulimia: Although bulimic women often maintain a normal weight, they do so by restricting food intake when not bingeing and purging, and they may have monotonous and poorly balanced meals. Starvation-induced depletion of hepatic glycogen stores results in free fatty acids and ketone bodies replacing glucose as the primary energy source. This shift from glycogenolysis to lipolysis and ketogenesis is associated with an increase in free fatty acids and their metabolites. β-Hydroxybutyric acid levels are elevated in both anorexia and bulimia nervosa [72], indicating that bulimic patients are nutritionally depleted in spite of their normal body weight.

The relationship of starvation and eating disorders to neuroendocrine function is most clearly seen for the pituitary-gonadal axis. Secondary amenorrhea is one of the criteria for anorexia nervosa in postmenarcheal women, and oligomenorrhea occurs in about 50% of bulimics. The secondary amenorrhea is a direct result of altered gonadotropin secretion. Serum sex hormone binding globulin may be increased, and both estrogen and testosterone are decreased [73]. The luteinizing hormone response to luteinizing hormone-releasing hormone stimulation is blunted, but the follicle-stimulating hormone response is usually normal. The amenorrhea in AN is related to deficient and dysrhythmic hypothalamic gonadotropin releasing hormone (GnRH) release. Although the degree of immature pattern of luteinizing hormone (LH) scretion does not correlate to the extent of weight loss [74], it is well documented that a critical minimum body weight is necessary for the onset and maintenance of normal menstrual cycles [75]. In fat tissue androgens are aromatized to oestrogens. Thus the poor body fat mass in AN undoubtedly contributes to hypooestrogenaemia, and consequently failing positive feed-back stimulation of the gonadotropins.

With reference to the hypothalamic-pituitary-adrenal cortical (HPA) axis, it is well known that plasma cortisol is increased at all times of the day and night, but its circadian rhythm is preserved in terms of amplitude and timing. Stimulation and suppression tests of the HPA axis have been conducted mainly in anorexia nervosa, and they are in accord with the baseline hormone findings. Adrenocorticotropic hormone (ACTH) response to corticotropin-releasing hormone (CRH) administration is reduced, undoubtedly secondary to enhanced negative feedback on the pituitary corticotrophs exerted by elevated circulating cortisol. The cortisol response to ACTH administration is increased, suggesting increased secretory capacity of the adrenal cortex. The low-dose dexamethasone suppression test is abnormal in 50% to 90% of anorexics and in 20% to 60% of bulimics, depending on the weight loss. Because dexamethasone acts primarily at the pituitary, ACTH and cortisol escape from dexamethasone suppression, suggesting increased suprapituitary stimulation of corticotrophs by CRH and vasopressin. Taken together, the pituitary-adrenocortical findings indicate a mild to moderate activation of this hormone axis in anorexia and bulimia nervosa. Interestingly, the abnormalities in anorexia nervosa and in reduced-weight bulimia nervosa [13,76] are strikingly similar to those occurring in 30% to 50% of patients with major depression, although malnutrition, and not mood disturbances, are likely to be most contributory.

With reference to the pituitary-thyroid axis, starvation leads to considerably decreased plasma free triiodothyronine (T_3) concentrations, along with somewhat decreased plasma free thyroxine (T_4) and increased plasma reverse T_3 concentrations. This represents the "euthyroid sick syndrome" hormone profile [77,78]. The decreased circulating T_3 helps reduce energy expenditure and minimizes muscle protein catabolism into amino acids for gluconeogenesis. CSF thyreotropin-releasing hormone also appears to be reduced in anorexia nervosa [79]. When bingeing, bulimic patients generally have normal thyroid indices with perhaps reduced T_3 and thyroid-stimulating hormone concentrations; however, when they become abstinent, their pituitary-thyroid axis function resembles that of anorexic patients [80-82].

Insulin-like growth factor, type I (IGF-1) concentrations are low in both anorexia nervosa and bulimia nervosa, and circulating growth hormone is increased, perhaps owing to diminished feedback of IGF-1 on growth hormone secretion. Circulating prolactin is usually unchanged in anorexia nervosa and may be reduced in bulimia nervosa. Prolactin responses to serotonergic challenges such as meta-chlorophenylpiperazine, fenfluramine, L-tryptophan, and 5-OH-tryptophan are diminished in both anorexia and bulimia nervosa.

CONCLUSIONS AND PERSPECTIVES

The increase in understanding of neuropeptide modulation of appetite and weight control also resulted in new insights into endocrine and neuropepetide disturbances in AN and BN. Obviously, there are still many methodological problems which have to be taken into consideration when interpreting the endocrinologic observations. Animal models which focus on one facet of behavior, such as motor activity or sexual receptivity, are not necessarily suitable models for AN. The serum concentrations of monoamines and peptides reflect pituitary secretion, but may provide a limited perspective on higher brain function. CSF measures reflect some general pool of chemicals, but offers limited understanding of specific pathways. Minor weight changes in patients with AN are

associated with significant responses in serum catecholamines, leptin, cortisol, gonadotropin and GH, indicating that the timing of the respective investigations is of critical importance and may be a cause of discrepant findings in several studies [4].

Determining whether abnormalities are a consequence or a potential antecedent of pathological feeding behavior is a major question in the question of eating disorders. When studying patients who had recovered from their eating disorder, any persistent psychobiological abnormalities might be trait-related and potentially have contributed to the pathogenesis of the disorder.

And last but not least, most models describe only one or two specific systems out of many, while our knowledge about the interactions between these systems is limited and, at the present time, it is not possible to map the sum of numerous interactive pathways.

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