Neurotransmitter and Imaging Studies in Anorexia Nervosa: New Targets for Treatment

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Abstract: Anorexia and Bulimia Nervosa are disorders of unknown etiology that invariably begin during adolescence and near in time to puberty in young women. These disorders are associated with aberrant eating behaviors, body image distortions, impulse and mood disturbances, as well as characteristic temperament and personality traits.

It is well known that malnutrition produces changes in neuroendocrine function. More recently, disturbances in neuronal systems have been found to play a role in the modulation of feeding, mood, and impulse control. These neuronal systems include neuropeptides (CRH, opioids, neuropeptide-Y (NPY) and peptide YY (PYY), vasopressin and oxytocin, CCK, and leptin) and monoamines (serotonin, dopamine, norepinephrine). Disturbances of most of these neuronal systems have been found when people are ill with an eating disorder, but it was not certain whether they were a cause or consequence of symptoms. In order to address these questions, a growing number of studies have investigated whether neuromodulatory disturbances persist after recovery. Studies from several centers tend to show altered serotonin activity persists after prolonged normalization of weight, nutrition, and menstrual function, as do anxiety, obsessionality, and perfectionism. While there are fewer data, there may be persistent alterations of dopamine or some neuropeptides in some subjects in a recovered state.

The inaccessibility of the central nervous system has made it difficult to understand brain and behavior. In the past decade, new tools, such as brain imaging, have offered the possibility of better characterization of complex neuronal function and behavior. Such studies have tended to consistently find that alterations of brain regions, such as the temporal lobe, occur in people who are ill with anorexia nervosa and appear to persist after some degree of weight gain and recovery. New imaging technology, that marries Positron Emission Tomography (PET) imaging with selective neurotransmitter radioligands, confirms that altered serotonin neuronal pathway activity persists after recovery from an eating disorder and supports the possibility that these psychobiological alterations might contribute to traits, such as increased anxiety or extremes of impulse control, that, in turn, may contribute to a vulnerability to the development of an eating disorder. In summary, studies of pathophysiology are starting to nominate new candidates for treatment leading to the possibility of finding effective treatments for this often chronic or fatal disorder.

Key words: anorexia nervosa, eating disorders, neurotransmitters, monoamines, serotonin, dopamine, neuroimaging, treatment

1. INTRODUCTION

Anorexia nervosa (AN) and bulimia nervosa (BN) are disorders characterized by aberrant patterns of eating behavior and weight regulation with disturbances in attitudes and perceptions towards shape and weight. Individuals with AN exhibit characteristic features, including an unrelenting obsession with weight loss and an inexplicable fear of fatness, even in the face of increasing cachexia [1]. Variations in feeding behavior have been used to subdivide individuals with AN into two meaningful diagnostic subgroups [1] that have been shown to differ in other psychopathological characteristics [2]. In the restrictor subtype of AN, subnormal body weight and an ongoing malnourished state are maintained by unremitting food avoidance. In the bulimic subtype of AN, there is comparable weight loss and malnutrition, yet the course of illness is marked by supervening episodes of binge eating, usually followed by some type of compensatory action such as self-induced vomiting or the misuse of laxatives, diuretics, or enemas.

Individuals with the bulimic subtype of AN are more likely to exhibit histories of behavioral dyscontrol, substance abuse, and overt family conflict in comparison to those with the restricting subtype. Also particularly common in individuals with AN are personality traits of marked

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perfectionism, conformity, obsessionality, constriction of affect and emotional expressiveness, and reduced social spontaneity; these traits typically appear in advance of the onset of illness and persist even after long-term weight recovery, indicating they are not merely epiphenomena of acute malnutrition and disordered eating behavior [3-5].

Individuals with BN remain at normal body weight during the course of illness, although many aspire to ideal weights far below the range of normalcy for their age and height. The core features of BN include repeated episodes of binge eating followed by compensatory self-induced vomiting, laxative abuse, or pathologically extreme exercise, as well as abnormal concern with weight and shape. The DSM-IV [1] has specified a distinction within this group between those individuals with BN who engage in self-induced vomiting or laxative, diuretic, or enema abuse (purging type), and those who exhibit other forms of compensatory action such as fasting or exercise (nonpurging type). Beyond these differences, it has been speculated [6] that there are two clinically divergent subgroups of individuals with BN differing significantly in psychopathological characteristics: a so-called multi-impulsive type, in whom BN occurs in conjunction with more pervasive difficulties in behavioral self-regulation and affective instability, and a second type whose distinguishing features include self-effacing behaviors, dependence on external rewards, and extreme compliance. Individuals with BN of the multi-impulsive type are far more likely to have histories of substance abuse and display other impulse control problems such as shoplifting and self-injurious behaviors. Considering these differences, it has been postulated that multi-impulsive BN individuals rely on binge eating and purging as a means of regulating intolerable states of tension, anger, and fragmentation; in contrast, individuals with BN of the latter type may have binge episodes precipitated through dietary restraint with compensatory behaviors maintained through reduction of guilty feelings associated with fears of weight gain.

Recovery from AN tends to be protracted, but roughly 50% of individuals will eventually have reasonably complete resolution of the illness, whereas another 30% will have lingering residual features that wax and wane in severity long into adulthood. Once developed, AN will pursue a chronic, unremitting course in some 10% of individuals and the remaining 10% of those affected will eventually die from the disease [7]. For BN, follow-up studies 5 to 10 years after presentation showed a 50% rate of recovery while nearly 20% continued to meet full criteria for BN [8]. Following onset, disturbed eating behavior will wax and wane over the course of several years in a high percentage of clinic cases. Approximately 30% of women who had been in remission experienced relapse into bulimic symptoms, although risk of relapse appeared to decline 4 years after presentation [8].

2. BIOLOGICAL RATIONALE

While the etiology of AN is unknown, it is assumed to be multifactorial and complex. Research has focused on the relative influences of genetic, biological, and psychosocial factors that may contribute to the onset of an eating disorder.

Although it has been argued that cultural attitudes towards standards of physical attractiveness have relevance to the psychopathology of AN, evidence suggests cultural influences are unlikely to be prominent risk factors. First, dieting behavior and drive for thinness are common practices in industrialized countries throughout the world, yet AN affects only an estimated 0.3% to 0.7% of females in the general population [1]. Second, clear case descriptions of AN date back to the middle of the 19th century, which suggests that factors other than a cultural emphasis on thinness play an etiologic role [9]. In addition, the relatively stereotypic clinical presentation, sex distribution, and age of onset in AN provides support for the possibility of some biological vulnerability to the disorder.

AN is associated with a range of psychological symptoms aside from pathological eating behaviors including depression, anxiety, and obsessionality. Individuals with AN also endorse stereotypical personality features including marked rigidity, overcontrol, and perfectionism [4-6, 10] which are present during the acute phase of illness and remain following long-term recovery. Determining whether these symptoms are a consequence or a potential cause of pathological feeding behaviors and malnutrition is a major methodological issue in the field of eating disorders. It is impractical to study AN prospectively due to the young age of onset and difficulty in premorbid identification of individuals who will develop an eating disorder. Alternatively, individuals may be studied after long-term recovery under the assumption that in the absence of the confounding effects of malnutrition, persistent psychobiological abnormalities may be trait related and contribute to the etiology of the disorder. Moreover, while the definition of recovery from AN has not been formalized, researchers tend to utilize a definition which includes a stable, healthy body weight, the resumption of menses, and the absence of disordered eating behavior for a period of at least one year.

Researchers have reported that individuals who are long-term recovered from AN had a persistence of obsessional behaviors, inflexible thinking, restraint in emotional expression, and a high degree of self- and impulse control [3-5]. Moreover, these individuals tend to have social introversion, overly compliant behavior, limited social spontaneity, and greater risk and harm avoidance. Drive for thinness and significant psychopathology related to eating habits also continued to be endorsed following long-term recovery from AN.

3. IMAGING STUDIES

3.1 Brain Imaging Studies

It is well known that ill AN subjects have enlarged ventricles and sulci widening (see review [11]). 1H-MRS revealed reduced lipid signals in the frontal white matter and occipital gray matter, and was associated with decreased body mass index [12]. These alterations have been thought to be reversible after recovery but recent data suggest persistent changes after recovery [13, 14].

3.2 "Resting" Studies

Most studies that have assessed "resting" brain activity in AN have used single photon computed tomography (SPECT). Gordon [15] found that 13 of 15 ill AN had unilateral temporal lobe hypoperfusion that persisted in the subjects studied after weight restoration [16]. Kuruoglu et al [17] found 2 ill AN had bilateral hypoperfusion in frontal, temporal, and parietal regions which normalized after 3 months of remission. Takano et al [18] found hypoperfusion in the medial prefrontal cortex and anterior cingulate and hyperperfusion in the thalamus and amygdala-hippocampus complex. Rastam et al [19] found temporoparietal and orbitofrontal hypoperfusion in ill and recovered AN (and AN-BN) subjects. One study, using PET FDG [20] showed AN had global hypometabolism that was most prominent in frontal and parietal regions. In summary, all SPECT studies have shown temporal alterations and most have shown frontal or cingulate or parietal changes. Importantly, the groups that have imaged AN subjects after some degree of recovery show persistence of temporal alterations.

3.3 "Activated" Studies

Several groups have used designs that activate symptoms in AN. After eating, ill AN had an increase in temporal, parietal, and occipital regions on SPECT compared to controls [21]. Ellison [22], using fMRI, found that ill AN subjects, when viewing pictures of high caloric drinks, had increased signal changes in the left insular, cingulate gyrus, and left amygdala-hippocampus region and increased anxiety. Gordon [23] using PET O-15 and pictures of high calorie food, found ill AN subjects had elevated cerebral blood flow (rCBF) in bilateral medial temporal lobes and increased anxiety. Naruo [24], who used SPECT to investigate imagining food, found that ill binge-eating/purging type AN subjects that had a significantly higher percent change in the inferior, superior, prefrontal, and parietal regions of the right brain than restricting type AN subjects or controls as well as the highest level of apprehension in regard to food intake. Seeger et al [25] using fMRI and a computer-based image distortion technique, found individuals with AN had activation of the R amygdala, R gyrus fusiformis, and brainstem regions, suggesting involvement of the brains "fear" network. An activation study [21] in ill BN using SPECT found feeding reduced temporal activity, which was in contrast to the marked increase in cortical activity found in AN after feeding. In summary, many of these studies showed that various food-related challenges activated temporal regions in AN compared to controls, and were associated with anxiety. These findings are remarkable considering the small number of subjects studied.

Most resting and activation brain imaging studies have show temporal region disturbances in AN subjects when ill and after various degrees of recovery. In addition, other brain areas, such as frontal and cingulate regions, and the parietal cortex are often altered. While these studies show remarkable consistency, particularly in terms of temporal involvement, it should be noted that numbers of subjects in each study tends to be small, and there is often inconsistency in terms

of definition of subgroups and states of illness studied. Small sample size and irregular definitions makes it difficult to know whether these are lateralizing findings or there are differences between subtypes of eating disorders. The meaning of these findings is open to interpretation. At the least, they suggest that regions of the brain involved in the modulation of mood, cognition, impulse control, and decision making may be altered in AN. Still, this is a promising start and should be strong support for further investigations.

4. 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 [26, 27]. 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 [28, 29].

While there are relatively few studies to date, most of the neuroendocrine and neuropeptide alterations apparent during symptomatic episodes of AN 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 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 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.

4.1 Corticotropin Releasing Hormone (CRH)

When underweight, individuals with AN have increased plasma cortisol secretion that is thought to be at least in part

a consequence of hypersecretion of endogenous CRH [30-33]. In that the plasma and cerebrospinal fluid (CSF) measures return toward normal, it appears likely that activation of the hypothalamic-pituitary-thyroid 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 [34].

4.2 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 [35]. State-related reductions in concentrations of CSF beta-endorphin and related opiate concentrations have been found in underweight AN subjects [36-38]. In contrast, using the T-lymphocyte as a model system, Brambilla *et al.* [39] found elevated beta-endorphin levels in AN. 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.

4.3 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 [27, 35, 40]. PYY is more potent than NPY in stimulating food intake; both are selective for carbohydrate rich foods. Underweight AN individuals have been shown to have elevations of CSF NPY, but normal PYY [41]. Clearly, elevated NPY does not result in increased feeding in underweight AN individuals; however, the possibility that increased NPY activity underlies the obsessive and paradoxical interest in dietary intake and food preparation is a hypothesis worth exploring. More recently, it has been reported that the plasma concentration of NPY was lower in AN patients than in controls [42]. Additional studies will be needed to assess the potential behavioral correlates of these findings.

4.4 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 [43]. In parallel to its role in satiety in rodents, exogenously administered CCK reduces food intake in humans. Studies of CCK in AN have yielded inconsistent findings. Some studies have found elevations in basal levels of plasma CCK [44, 45], as well as increased peptide release following a test-meal [44, 46]. One study found that blunting of CCK response to an oral glucose load normalized in AN patients after partial restoration of body weight [45]. Other studies have found that measures of CCK

function in AN were similar to or lower than control values [47-49]. Further studies are needed to evaluate the relationship between altered CCK regulation and other indices of abnormal gastric function in symptomatic AN individuals.

4.5 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. In humans, serum and CSF concentrations of leptin are positively correlated with fat mass in individuals across a broad range of body weight, including obesity [50, 51]. 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 [52].

Underweight patients with AN have consistently been found to have significantly reduced serum leptin concentrations in comparison to normal weight controls [42, 53-56]. 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 [56]. 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 patients with AN may be higher than expected based on the extent of weight loss [57, 58]. Mantzoros *et al.* [56] 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 individuals with AN 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 [56]. 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 [59].

5. NEUROTRANSMITTERS

A role for biological determinants in the pathogenesis of eating disorders has been proposed for the past 60 years [9]. In particular, an increased knowledge of the neurotransmitter modulation of feeding behavior has raised questions as to whether a disturbance in monoamine function may play a role in these disorders.

5.1 Serotonin (5-HT)

Serotonin pathways play an important role in postprandial satiety. Treatments that increase intrasynaptic 5-HT, or directly activate 5-HT receptors, tend to reduce food

Table 1.   Metabolic Pathway of Serotonin and Dopamine Synthesis

tryptophan	tyrosine
↓ tryptophan-5-hydroxylase	↓ tyrosine hydroxylase
5-hydroxytryptophan	dopa
↓ aromatic amino acid decarboxylase	↓ aromatic amino acid decarboxylase
5-hydroxytryptamine (5-HT)	dopamine
↓ monoamine oxidase	
5-hydroxyindoleacetic acid	

consumption whereas interventions that dampen serotonergic neurotransmission or block receptor activation reportedly increase food consumption and promote weight gain [60, 61]. Moreover, CNS 5-HT pathways have been implicated in the modulation of mood, impulse regulation and behavioral constraint, and obsessionality, and they affect a variety of neuroendocrine systems.

Serotonin is synthesized from its precursor tryptophan, an essential amino acid that must be obtained through the diet (Table 1). Following dietary consumption, tryptophan is taken up by the brain and hydroxylated by the enzyme tryptophan-5-hydroxylase [62]. The product of this reaction, 5-hydroxytryptophan, is then decarboxylated by aromatic amino acid decarboxylase to the compound 5-hydroxytryptamine. Monoamine oxidase further metabolizes 5-hydroxytryptamine to the metabolite product known as 5-hydroxyindoleacetic acid (5-HIAA), which may be measured as a means of assessing 5-HT turnover or metabolism [62].

There has been considerable interest in the role that 5-HT may play in eating disorders [9, 63-66]. In part this is related to the fact that studies have found that individuals with AN have alterations in 5-HT metabolism. During the acute phase of illness, a significant reduction in basal levels of CSF 5-HIAA has been found in individuals with AN compared to healthy controls [67]. Although one study did not find a significant difference between groups [68], subjects were assessed after a period of weight gain and nutritional restoration. Concentrations of CSF 5-HIAA have been found to normalize with weight gain [69], and thus reduced concentrations of CSF 5-HIAA during the acute phase of AN may reflect a consequence of starvation. Researchers have also reported a blunted plasma prolactin response to serotonergic agonists and reduced ³H-imipramine binding in these individuals. Taken together, these findings suggest reduced serotonergic activity in AN during the acute phase of illness.

Levels of CSF 5-HIAA have been found to be significantly elevated following a period of long-term

recovery in AN [70]. Moreover, some but not all studies using challenges, such as tryptophan depletion and m-CPP, suggest such interventions may reduced dysphoric mood in people who are recovered from AN [71, 72]. Increased serotonergic activity has been implicated in anxious and obssessive behavior in humans and animals which are also symptoms that persist after recovery from AN. Thus, it can be argued that persistent alterations in the modulation of 5-HT during the recovered state may play a role in the persistence of certain behavioral traits including overly inhibited, anxious, and obsessional behavior [70, 73].

It is important to note that the 5-HT system is complex, involving several brain stem nuclei, multiple pathways, different regions and innervations, 14 or more receptors, and many other metabolic and intracellular components. Attempting to characterize such complexity by the use of CSF 5-HIAA or challenge studies is not possible – such studies merely serve as a means of reflecting some aberrations of this system. Fortunately, more powerful tools may offer the possibility of better characterization of complex neuronal function and behavior. The marriage of Positron Emission Tomography (PET) imaging with selective neurotransmitter radioligands has resulted in a technology permitting new insights into regional binding and specificity of 5-HT and dopamine neurotransmission in vivo in humans and their relationship to behaviors.

Our group has used this technology to study women after recovery from AN and BN (>1 year no bingeing or purging, normal weight, and regular menstrual cycles) to confirm disturbances in 5-HT activity and provide new insights into the disorders. PET and [^{18}F]altanserin was used to study women who were recovered from restricting-type AN [74]. Recovered restricting-type AN women had reduced 5-HT$_{2A}$ activity, relative to control women, in mesial temporal (amygdala and hippocampus) regions, as well as cingulate, sensorimotor, and occipital/parietal cortical regions.

5-HT$_{1A}$ receptor activity was investigated in recovered AN women compared to control women [75] using PET

with [carbonyl-^{11}C]WAY100635, a specific 5-HT$_{1A}$ receptor antagonist. Individuals recovered from AN had a 30 to 60% increase of 5-HT$_{1A}$ receptor activity in the raphe nucleus (pre-synaptic 5-HT$_{1A}$ autoreceptors) and cortical-limbic-striatial regions (postsynaptic 5-HT$_{1A}$ receptors). Moreover, 5-HT$_{1A}$ postsynaptic receptor binding in many cortical regions was positively correlated with trait anxiety and harm avoidance in the AN group. Pharmacological and knock out studies implicate the 5-HT$_{1A}$ receptor in the modulation of anxiety [76]. Anxiety is a common premorbid trait in people who develop AN [77-79]. Moreover, anxiety symptoms invariably occur in ill AN individuals and persist after recovery [4]. Finally, the depletion of tryptophan, the precursor of serotonin, specifically reduces anxiety, but does not affect other mood states in ill and recovered AN women [72].

This technology holds the promise of an era of understanding the complexity of neuronal systems in human behavior. For example, post-synaptic 5-HT$_{1A}$ receptors [80-83] have "downstream" effects and interactions with other neuronal systems, such as norepinephrine, glutamate, and GABA. Enhanced 5-HT$_{1A}$ activity in AN may cause or reflect an altered balance between these neuronal systems. Moreover, 5-HT$_{1A}$ receptors interact with other 5-HT receptors such as 5-HT$_{2A}$ [82, 84]. Our preliminary data found an inverse relationship between post-synaptic 5-HT$_{1A}$ and 5-HT$_{2A}$ receptor activity in AN. 5-HT$_{1A}$ post-synaptic receptors mediate locus coeruleus firing through 5-HT transmission at 5-HT$_{2A}$ receptors [82]. Theoretically, increased 5-HT$_{1A}$ and reduced 5-HT$_{2A}$ post-synaptic receptor activity in AN might result in an increase in noradrenergic neuron firing [82]. Moreover, post-synaptic 5-HT$_{1A}$ receptors hyperpolarize and 5-HT$_{2A}$ receptors depolarize layer V pyramidal neurons [85]. In AN, synergistic effects of these receptors, which are co-localized on pyramidal neurons, may reduce pyramidal neuronal excitability.

In summary, these PET-radioligand studies confirm that altered 5-HT neuronal pathway activity persists after recovery from AN and support the possibility that these psychobiological alterations might contribute to traits, such as increased anxiety, that may contribute to a vulnerability to develop an eating disorder.

A major point of interest in the study of 5-HT modulation and its relation to eating disorders is the enzyme that catalyzes the rate-limiting step in 5-HT synthesis. Since the enzyme tryptophan hydroxylase is not normally saturated by tryptophan, the rate of 5-HT synthesis can be influenced by changes in brain tryptophan concentration [86]. This concentration is dependent upon the plasma concentration of tryptophan as well as the ratio of tryptophan to other large neutral amino acids with which it competes for uptake [87]. Thus, the concentration of tryptophan available for 5-HT synthesis is influenced by the relative concentrations of dietary intake of amino acids.

Dieting for as little as three weeks has been found to decrease plasma tryptophan levels in healthy individuals [88]. This decrease in plasma tryptophan was more severe in women than in men, despite a similar percentage of weight loss. Moreover, a marked increase in prolactin response has been found following intravenous administration of L-tryptophan in dieting women but not in dieting men [89]. These findings are of particularly importance in the study of risk factors for eating disorders, given that 90% to 95% individuals that develop AN are female [1].

Given that food restriction is not an inherently reinforcing behavior in healthy individuals, persistent dieting to the point of starvation suggests that food restriction may have some intrinsic benefit for individuals with AN. The ratio of tryptophan to other large neutral amino acids has been found to be significantly reduced in AN [90]. This reduction is likely to be a consequence of starvation since food restriction results in a decrease in dietary tryptophan consumption and thereby a decrease in the concentration of tryptophan available for 5-HT synthesis.

Premorbidly, individuals with AN report high levels of anxiety and obsessionality. Evidence suggests that individuals with AN may have an intrinsic defect in the 5-HT system. These individuals may have high levels of 5-HT in the synapse premorbidly resulting in a dysphoric state. Dieting may serve as a means of regulating this overactivity of 5-HT by decreasing the amount of tryptophan available for 5-HT synthesis, as evidence of reduced 5-HT activity is found during the acute phase. A recent study of acute tryptophan depletion found that a reduction of dietary tryptophan was associated with decreased anxiety and an elevation of mood in individuals with AN during the acute phase of illness and following long-term recovery [72]. Acute tryptophan depletion did not have significant anxiolytic effects for control women.

5.2 Dopamine

Altered dopamine activity has been found among ill AN individuals. Dopamine is synthesized from its amino acid precursor tyrosine (Table 1). Homovanillic acid (HVA), the major metabolite of dopamine in humans, was decreased in CSF of underweight AN subjects [67]. Our group found [91] that recovered AN subjects had significantly reduced concentrations of CSF HVA, compared to recovered BN-AN or BN women. Dopamine neuronal function has been associated with motor activity [91], reward [92, 93], and novelty seeking. Individuals with AN have stereotyped and hyperactive motor behavior, anhedonic, restrictive personalities, and reduced novelty seeking. Whether individuals with AN have an intrinsic disturbance of dopamine remains uncertain.

6. PHARMACOLOGIC TREATMENT OF AN

Most medication trials in AN have been conducted with inpatients in an attempt to accelerate restoration of weight. Some studies also examined the impact of medication on mood or anorectic attitudes. A wide variety of psychoactive medications, such as L-dopa [94], phenoxybenzamine [95], diphenylhydantoin [96, 97], stimulants [98], and naloxone [99], have been administered to individuals with AN in open, uncontrolled trials. In many of these trials, medications have been claimed to be beneficial, but none of

these observations has been confirmed under double-blind, controlled conditions.

Relatively few studies of medication using rigorous double-blind placebo-controlled trials have been reported in individuals with AN. In contrast to the positive claims from open trials, results from double-blind trials have been, for the most part, limited. Double-blind studies, at most, report marginal success in treatment of specific problems such as improving the rate of weight gain during refeeding, disturbed attitudes towards food and body image, depression, or gastrointestinal discomfort.

One problem with determining the efficacy of pharmacotherapy in AN is that often medications have been given in association with other therapies. Thus, it may be unclear whether it was the medication or therapy that resulted in improvement. Furthermore the primary criterion for improvement has often been weight gain, not a normalization of thinking and a reduction in fears of being fat. It is important to emphasize that treatment in structured settings, such as inpatient units, even without medication, succeeds in restoring the weight of over 85% of underweight patients [100]. Thus, it may be difficult to prove that an active medication is effective in such a setting. However, relapse within one year after successful inpatient weight restoration is very common [101]. For example, the Maudsley study [102] reported that only 23% of patients had a good outcome at one year after discharge despite intensive outpatient individual or family therapy.

Controlled trials of the neuroleptics pimozide [103] and sulpiride [104] have suggested limited effects in accelerating weight gain or altering anorectic attitudes for some patients for part of the study, but overall drug effect was marginal. Recently, there has been clinical interest in atypical neuroleptics for AN because of their notoriety for causing weight gain in other patient populations [105]. A recent case report suggested that olanzapine administration was associated with weight gain and maintenance as well as reduced agitation and resistance to treatment in 2 women with AN [106]. Olanzapine is a novel, atypical antipsychotic drug that interacts with dopaminergic, serotonergic, adrenergic, and muscarinic receptors [107], thus the neuronal systems responsible for the drug's potential efficacy remain uncertain. Considerable evidence from animal and human studies has implicated these systems in modulation of feeding behavior, mood, and obsessionality. A number of recent uncontrolled studies have provided preliminary evidence that olanzapine may be helpful in facilitating weight gain and decreasing levels of anxiety and depression during the treatment of AN [108-112].

Several drugs have been tested because of anecdotal reports of their effects on stimulating appetite. Tetrahydrocannabinol (THC) was not useful and, in fact, may have been detrimental as it increased dysphoria in some patients [113]. Clonidine was also found to have no therapeutic effect on increasing weight restoration as compared to placebo [114] even with doses that effected hemodynamic parameters. When underweight, patients with AN have delayed gastric emptying [115] which improves

with refeeding. Still, delayed gastric emptying could perpetuate the disorder in some patients by limiting the quantity of food that may be comfortably eaten. Most studies of prokinetic drugs in AN have been limited to parenteral preparations or to experiments with small uncontrolled groups of patients [116-118]. In a controlled trial, cisapride [119] was no better than placebo in improving gastric emptying although some subjective measures of distress during meals and measures of hunger improved more in the group on cisapride.

In summary, these medication trials have been of short duration and have focused on whether medication produces additive benefit to an established treatment program. Few follow up studies have examined whether medication treatment produces lasting benefit. A new generation of studies has begun to focus on whether medication can prevent relapse after patients leave to a structured treatment setting.

6.1 Use of Antidepressants in AN

There has been controversy as to whether AN and major depressive disorders share a common diathesis. However, critical examination of clinical phenomenology, family history, antidepressant response, biological correlates, course and outcome, and epidemiology yield limited support for this hypothesis [120-122]. Still, the high frequency of mood disturbances associated with this disorder has resulted in trials of drugs such as amitriptyline [123-125], and lithium [126]. Neither medication appears to significantly improve mood compared with the effects of placebo.

For more that 50 years [127], investigators have suggested that AN shares similarities with obsessive-compulsive disorder (OCD). In fact, patients with AN have a high prevalence of obsessive-compulsive symptoms or disorders [5, 128, 129], as well other anxiety disorders [130]. More over, adult women with OCD have an increased incidence of prior AN [131, 132]. Individuals with a past history of AN display evidence of increased 5-HT [70] activity that persists after long-term weight-recovery. In addition, women who recover from AN continue to have modest, but significant, increases in negative mood, obsessionality, perfectionism, and core eating disorder symptoms. Similarly, personality characteristics associated with AN, such as introversion, self-denial, limited spontaneity, and a stereotyped thinking style, may also persist after weight recovery. Studies in humans and animals suggests that 5-HT activity is related to behavioral inhibition. Together, these data raise the possibility that increased CSF 5-HIAA could be associated with inhibition and an obsessive need with exactness and perfectionism. A disturbance of this neurotransmitter system has been implicated in OCD [133] and only serotonin-specific medication has been found to be useful in treating OCD.

There are suggestions that medications which affect the 5-HT system may impact the clinical characteristics of patients with AN. Initial reports on cyproheptadine, a drug that is thought to act on the serotonergic and histaminergic

systems [134], indicated that it might have beneficial effects on weight gain, mood, and attitude in some patients [135, 136]. Cyproheptadine data from comparison trials with amitriptyline and placebo found cyproheptadine to significantly improve weight gain in the restricting subtype of AN, while amitriptyline was more effective in those patients with bulimic behavior [137].

Several groups [138, 139] reported that an open trial of fluoxetine, a highly specific serotonin reuptake inhibitor (SSRI) may help patients with AN gain and/or maintain a healthy body weight. Recently, the Pittsburgh group reported a double-blind placebo-controlled trial of fluoxetine in 35 patients with restrictor-type AN [140]. Subjects were started on fluoxetine after they achieved weight restoration (approximately 90% of ideal body weight) during a hospitalization. Patients were randomly assigned to fluoxetine (n = 16) or placebo (n = 19) after inpatient weight-restoration and then were followed as outpatients for one year. After 1 year of outpatient follow-up, 10 of 16 (63%) subjects had a good outcome on fluoxetine whereas only 3 of 19 (16%) had a good outcome on placebo (p = .006). Aside from improved outcome, fluoxetine administration was associated with a significant reduction in obsessions and compulsions and a trend towards a reduction in depression. These data suggest that fluoxetine may help prevent relapse in some patients with AN.

It is important to note that SSRIs appear to have little effect on reducing symptoms and preventing hospitalization in malnourished, underweight AN individuals [141, 142]. Women with AN, when malnourished and underweight, have reduced plasma tryptophan availability [143] and reduced CSF 5-HIAA [69], the major metabolite of 5-HT in the brain. In addition, low estrogen values during the malnourished state may reduce 5-HT activity by effects on gene expression for 5-HT receptors [144] or the 5-HT transporter [145]. SSRIs are dependent on neuronal release of 5-HT for their action. If malnourished AN individuals have compromised release of 5-HT from presynaptic neuronal storage sites and reduced synaptic 5-HT concentrations, then a clinically meaningful response to an SSRI might not occur [146]. The possibility that fluoxetine is only effective for individuals with AN after weight restoration is supported by the fact that a change in 5-HT activity is associated with weight gain. For example, CSF 5-HIAA levels are low in underweight anorexics, normal in short-term weight-restored anorexics, and elevated in long-term weight-restored anorexics [67]. If CSF 5-HIAA levels accurately reflect CNS 5-HT activity, then these data imply that a substantial increase in 5-HT activity occurs after weight gain.

The use of serotonin-specific medications in the treatment of AN is promising but many questions remain. First, only one double-blind placebo-controlled study has been completed in a relatively small number of restrictor-type AN patients. Thus it will be important to replicate this work in a larger group of patients. Second, more data are needed to determine if there are differential effects in the restricting or binge eating/purging subtypes of AN. Third, it needs to be determined whether certain symptoms are especially responsive to serotonin-specific medications: core anorexic symptoms, depression, anxiety, obsessionality, or eating behavior.

6.2 Guidelines for Clinical Treatment

The first line of treatment for underweight patients with AN should be refeeding and weight restoration. As noted above, while difficult, most patients will gain weight in a structured eating disorders treatment program without the use of medication. Weight gain alone tends to reduce exaggerated obsessionality and dysphoric mood in many patients [147]. There is limited evidence that fluoxetine and possibly other serotonergic medications help prevent relapse after weight restoration. It is important to emphasize that some physiological and cognitive alterations persist for months after achieving goal weight. These include increased energy needs, menstrual disturbances, several neurotransmitter disturbances, urges to engage in disordered eating patterns, and body image distortions. Thus, treatment should continue for at least 3 to 6 months after achieving goal weight, preferably until there is resumption of menstrual periods, normalization of caloric needs, remediation of any physical complications, and sufficient remission of pathological eating and body-image distortions so that daily activities are not disturbed. We strongly support use of the recent American Psychiatric Association (APA) guidelines for eating disorders [148] which describes comprehensive treatment of AN.

7. SUMMARY

The inaccessibility of the central nervous system has made it difficult to understand brain and behavior. In the past decade, new tools have become available that permit direct measurements of complex brain function and relationships to behavior. These tools are contributing to accelerating progress in understanding the pathophysiology of AN and thus should advance treatment design.

Measures, such as plasma levels of hormones thought to reflect brain activity, have provided important understanding of the effects of malnutrition on endocrine function, and have been used as an index of brain functional activity. Evidence from animal studies has stimulated the investigation of neuronal systems known to play a role in the modulation of feeding, mood, and impulse control. These neuronal systems include neuropeptides (CRH, opioids, NPY, PYY, CCK, and leptin) and monoamines (serotonin, dopamine, norepinephrine). Serotonin has received the most attention because there is a good "fit" between it's role in the brain and symptoms in AN and because 5-HT medication may have some efficacy. While overly simplified, there is evidence of reduced 5-HT activity in ill AN and increased 5-HT activity after recovery. Considerable evidence from humans and animals indicates that "low" 5-HT activity is associated with impulsive and non-premeditated aggressive behaviors. Behaviors found after recovery from AN, such as obsessionality with symmetry and exactness, anxiety, and perfectionism, tend to be opposite in character. Thus 5-HT may correlate with a spectrum of behavior spanning behavioral undercontrol to behavioral overcontrol.

It is well known that diet-induced changes in tryptophan, the precursor of 5-HT, effect brain 5-HT release.

Malnutrition may reduce tryptophan availability and thus may mask intrinsic abnormalities in 5-HT systems that mediate certain core behavioral or temperamental underpinnings of risk and vulnerability. It is important to note that the 5-HT system has multiple brainstem nuclei and pathways, 14 or more receptors, and consists of many other elements such as transporter, metabolic enzymes, intracellular messenger, etc. Moreover, 5-HT has complex and poorly understood interactions with many other neurochemical systems. Conventional tools provide a distant and overly simple snapshot of some activity that reflects this complex system. Fortunately, new, more powerful tools, such as brain imaging, offer the possibility of better characterization of complex neuronal function and behavior.

Studies of pathophysiology are starting to nominate new candidates for treatment. For example, will drugs acting on 5-HT_{1A} post-synaptic receptors be useful in reducing anxiety (or satiety or behavioral overcontrol) in AN? As new 5-HT-specific drugs become available, they should be assessed in individuals with eating disorders. Limited data suggests SSRIs are not effective in the ill state, but do have efficacy after recovery in reducing relapse. Pharmacological treatment studies need to address the influences of malnutrition on drug response. In addition, dopamine metabolism may be altered in restrictor-type AN. Recently, open trials suggest olanzapine, which interacts with dopaminergic, serotonergic, adrenergic, and muscarinic receptors, has therapeutic efficacy in ill AN. Controlled trials of atypical neuroleptics are warranted as well as animal and human studies investigating their mode of action in AN.

AN invariably begins during adolescence and near in time to puberty in young women. Several lines of evidence support the possibility that developmental factors, likely related to female gonadal steroids, may trigger this illness. Moreover, a substantial number of subjects recover in their 20s raising the possibility of normalization of developmental disturbances at that age. Treatment studies need to incorporate the biological influences of gender and developmental factors, and animal studies of these influences should be encouraged.

In summary, AN is often a chronic disorder requiring costly hospitalizations and has the highest mortality of any psychiatric disorder. A better understanding of behavioral and neurobiological traits is likely to contribute to more effective treatments.

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