Emerging role of autoantibodies against appetite-regulating neuropeptides in eating disorders

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Manuscript received and accepted June 15, 2008.

Abstract

Objective: Recent findings of autoantibodies directed against melanocortin peptides suggest that these autoantibodies may represent a source of variability in peptidergic signaling that can be responsible for altered appetite and emotion in eating disorders. However, it is still unknown if autoantibodies directed against some other appetite-regulating neuropeptides and peptide hormones exist in healthy human subjects and if these autoantibodies can regulate appetite and emotion.

Methods: We determined the presence of autoantibodies against some key appetite-regulating neuropeptides and peptide hormones in sera of human subjects and in rats, and used animal models to study the role of α-melanocyte-stimulating hormone autoantibodies in food intake and anxiety.

Results: Immunoglobulin G and A autoantibodies against α-melanocyte-stimulating hormone, neuropeptide Y, agouti-related protein, ghrelin, leptin, and some other neuropeptides or peptide hormones involved in appetite control were present in healthy humans and rats. Animal models including active and passive transfer showed that α-melanocyte-stimulating hormone autoantibodies are involved in the regulation of feeding and anxiety. Sequence homology was found between neuropeptides and proteins from some members of intestinal microflora, whereas germ-free rats showed altered levels of autoantibodies directed against several neuropeptides.

Conclusion: Autoantibodies directed against appetite-regulating neuropeptides and peptide hormones are emerging as important participants in the peptidergic mechanisms controlling motivated behavior. Furthermore, these autoantibodies could provide a link in the gut–brain axis and may represent new biological targets for the diagnosis and treatment of eating disorders. © 2008 Elsevier Inc. All rights reserved.

Keywords: Anorexia nervosa; Bulimia; Hypothalamus; Gut–brain axis; Neuropeptides; Autoimmunity; Microbiota

Introduction

Anorexia nervosa (AN) and bulimia nervosa (BN) are two main forms of eating disorders characterized by a lack of physiologic control of appetite and disturbed emotions including high anxiety. The etiology of eating disorders is believed to be multifactorial, which signifies that the exact biological mechanisms responsible for development of these disorders are still poorly understood [1]. Nevertheless, neuropeptides may appear as key molecules involved in the pathogenesis of eating disorders based on numerous data showing that alterations of central and/or peripheral neuropeptidergic signaling are accompanied by disturbed regulation of body weight, appetite, or emotion as has been presented during 9th neuropeptide Y (NPY) meeting and reviewed in this issue [2–9].
There is growing evidence implicating the immune system in normal brain functions and in neurologic disorders [10], where T-cells [11], proinflammatory cytokines [12,13], or autoantibodies (autoAbs) directed against neurotransmitter receptors [14,15] play important roles. This review summarizes recent data showing that the immune system might also be responsible for the appearance of eating disorders via altered production of autoAbs directed against neuropeptides involved in the regulation of appetite and emotion.

Initial finding of neuropeptide autoAbs in eating disorders

By applying sera from patients with eating disorders on rat brain sections and immunohistochemically detecting immunoglobulin (Ig) G binding, a distinct staining pattern characteristic for peptidergic neurons was found in the arcuate nucleus of the hypothalamus (Fig. 1). Using absorption of the patients’ sera with several neuropeptides, α-melanocyte-stimulating hormone (α-MSH) was identified as the molecule responsible for immunostaining of these arcuate neurons. The same study also identified autoAbs directed against adrenocorticotropic hormone (ACTH) and gonadotropin-releasing hormone in the sera of several patients with eating disorders [16]. The logical suggestion that α-MSH autoAbs could only be associated with bulimia by a neutralizing satiety effect of α-MSH was not confirmed, because α-MSH autoAbs were detected in restrictive AN and in BN. However, an increased incidence of α-MSH and ACTH autoAbs binding to the rat brain and pituitary by sera from patients with eating disorders versus controls suggested that these autoAbs could be relevant to the mechanism of both eating disorders [16]. The importance of finding of α-MSH autoAbs in subjects with eating disorders is emphasized by the fact that α-MSH in the brain integrates several behavioral modalities including appetite and emotion [17], whereas activation of melanocortin receptors appears as the final common pathway for satiety signaling [18]. In addition, a follow-up study in patients with eating disorders and healthy controls identified the presence of autoAbs directed against oxytocin (OT) and vasopressin (VP) in their sera [19].

Link between α-MSH autoAbs and psychopathologic traits in eating disorders

To determine if neuropeptide autoAbs are associated with clinical symptoms of eating disorders, serum levels of autoAbs directed against α-MSH, ACTH, OT, or VP were measured by enzyme-linked immunosorbent assay in patients with restrictive AN or BN who had been evaluated by the Eating Disorders Inventory-2 scale (EDI-2) [20]. Significant correlations between the total EDI-2 score and levels of autoAbs directed against α-MSH, but not against other neuropeptides, were found in patients with AN and with BN, and this correlation was positive in AN but negative in BN [19]. Within the EDI-2, the subscale values for “drive for thinness,” “bulimia,” and “ineffectiveness” also significantly correlated with levels of α-MSH autoAbs, suggesting that these autoAbs might be associated with the appearance of the core psychopathologic traits characteristic for eating disorders. Importantly, levels of IgM α-MSH autoAbs also showed correlations with the EDI-2 values, but these correlations were opposite in patients with AN versus those with BN, suggesting that the IgG and IgM classes of these autoAbs might have opposite properties with regard to α-MSH signaling. Furthermore, serum levels of α-MSH IgM, but not of IgG autoAbs, were elevated in restrictive AN, pointing to their pathogenic role in the reduction of appetite. Interestingly, levels of autoAbs directed against different neuropeptides displayed correlations between each other (Fig. 2), suggesting that changes in levels of these autoAbs could be triggered by a common factor. Furthermore, in contrast to the immunohistochemical detection, the serum presence of autoAbs directed against α-MSH, ACTH, OT, or VP was readily detected by enzyme-linked immunosorbent assay in healthy controls. This may signify that these autoAbs participate in thus far unknown physiologic mechanisms characteristic for peptidergic transmission. In fact, the phenomenon of autoAbs directed
against peptide messenger molecules has been previously reported in healthy subjects and in patients with autoimmune diseases by identification of autoAbs against insulin [21], vasoactive intestinal peptide [22], several cytokines [23], and nerve growth factor [24]. The origin and functional role of these autoAbs remain largely unexplored with the exception of insulin autoAbs that are associated with type 1 diabetes [25]. In addition, serum levels of ACTH autoAbs were linked to aggressive behavior in male subjects [26].

AutoAbs against appetite-regulating neuropeptides in healthy subjects

To test the generality of the phenomenon of autoAbs with regard to neuropeptides and peptide hormones involved in the regulation of body weight, appetite, and emotion, we studied in healthy females the serum presence of IgG and IgA autoAbs directed against 14 key peptides including leptin, insulin, peptide YY, ghrelin, NPY, agouti-related protein, galanin, orexin, melanin-concentrating hormone, α-MSH, ACTH, corticotropin-releasing hormone (CRH), OT, and VP. The presence of IgG and IgA autoAbs directed against these peptide messengers was found in the sera of all subjects (Fig. 3), supporting the hypothesis of the generality of this phenomenon [27]. Our recent data indicate that levels of these autoAbs are altered in different ways in AN versus hyperphagic obesity (S. O. Fetissov, unpublished data). Furthermore, the presence of the IgA class of neuropeptide autoAbs suggests that at least some of these autoAbs can be stimulated by luminal antigens including microbial proteins of the gut microflora [28].

Link between gut microflora and autoAbs against appetite-regulating neuropeptides

The natural presence in healthy subjects of autoAbs directed against neuropeptides points to a physiologic origin. We applied an in silico approach to study if microbial proteins from intestinal microflora are relevant to the production of such autoAbs. This hypothesis is valid because gut microbial antigens participate in the production of physiologic autoAbs common to all human subjects, such as agglutinins characteristic for the ABO blood groups [29]. The appearance of such cross-reacting autoAbs is possible based on the concept of molecular mimicry between microbial and self-proteins validated for several autoimmune diseases [30]. Moreover, proteins from some gut bacteria were shown to cross-react with autoAbs recognizing thyrotropin-stimulating hormone receptor [31]. Therefore, we searched the public National Center for Biotechnology Information (NCBI) database for the presence of a sequence homology between 14 key appetite-regulating peptides and proteins from bacteria, viruses, fungi, and archaea. We found several sequence homologies of at least five consecutive amino acids, qualifying for the concept of molecular mimicry [32], between all studied neuropeptides and microbial proteins derived from commensal or pathogenic micro-organisms.
For instance, the α-MSH peptide displayed homology with commensal Bacteroides and Escherichia coli strains, ACTH with Lactobacilli, and α-MSH and ACTH had homologies with pathogenic Helicobacter pylori. These results indicate that different microbial proteins may trigger the appearance of autoAbs cross-reacting with the same neuropeptide, but targeting its different epitopes, resulting in different affinities of interactions between the neuropeptide and its autoAbs.

Interestingly, sequence homologies for proteins of the same micro-organism were found in several neuropeptides, suggesting that this micro-organism might trigger simultaneous changes in autoAb production directed against these neuropeptides, possibly resulting in a specific pattern of alteration of several peptidergic systems. For instance, the sequence homologies for proteins from Candida albicans were present among α-MSH, ACTH, OT, and VP peptides, and this may be relevant to their co-ordinated change, as noticed in patients with eating disorders (Fig. 2).

To verify if the intestinal microflora is necessary for the production of autoAbs against neuropeptides, serum levels of IgG and IgA autoAbs directed against 14 appetite-regulating peptides were compared between germ-free and specific pathogen-free Sprague-Dawley rats. These autoAbs were detected in both groups of rats, but levels of agouti-related protein, melanin-concentrating hormone, and CRH IgA autoAbs were lower, whereas ghrelin IgG autoAbs were higher in germ-free versus specific pathogen-free rats [27]. These data show that gut microflora is not compulsory for the origin of neuropeptide autoAbs, suggesting their primary ontogenic origin, similar to some other naturally occurring autoAbs [33,34]. However, it appears that gut microflora can selectively modulate levels of some of these autoAbs.

**Animal models to study the role of neuropeptide autoAbs**

Because rats naturally display neuropeptide autoAbs, it is possible to study their role in the regulation of appetite and emotion. Moreover, in analogy to classic animal models of autoimmune diseases, the causative pathogenic role of neuropeptide autoAbs can be studied using active or passive transfer methodology.

Because α-MSH and ACTH are stress-related peptide hormones, their increased secretion during stress could result in the stimulation of the production of autoAbs. We used an anxiolytic stress model by repeated handling of rats and found that rats exposed to such stress produce more IgG autoAbs directed against these hormones. Importantly, increased levels of α-MSH autoAbs in rats exposed to repeated mild stress were associated with increased food intake and reduced anxiety during strong stress induced by food restriction [35]. Furthermore, a model of passive transfer (intracerebral injection) of α-MSH IgG autoAbs purified from blood of rats exposed to repeated mild stress into the hypothalamus of naive rats showed that these autoAbs produce acute bulimic and anxiolytic responses (H. Sinno et al., unpublished observations).

In another set of experiments, we studied the effects of immunization of female Lewes rats with α-MSH peptide. We found that immunized versus control rats did not have differences in body weight and food intake under *ad libitum* conditions, but during food restriction the immunized rats consumed significantly more food versus controls (Fig. 4). Acute peripheral administration of α-MSH peptide reduced food intake in immunized and control rats (Fig. 4), suggesting that the immunization does not alter the normal response...
to α-MSH to increase satiety. Interestingly, some immunized rats showed a decrease in α-MSH immunostaining in the arcuate neurons, whereas staining for agouti-related protein did not change (Fig. 5). These results suggest that immunization against a neuropeptide can selectively deplete the intracellular content of this peptide, supporting previous data showing that anti-CRH or anti-VP monoclonal antibodies can penetrate CRH- or VP-producing hypothalamic neurons, respectively [36]. Therefore, immunization with α-MSH may represent a putative new approach for the therapy or prevention of eating disorders to prevent the development of AN during dieting. In fact, a similar approach was recently reported for obesity treatment showing a decrease in body weight gain in rats immunized with fragments of ghrelin, an orexigenic peptide [37]. Moreover, as reviewed in detail in this issue of Nutrition, active immunization with the melanocortin-4 receptor also results in a reduced weight gain in rats [9], supporting a functional role of peripheral autoAbs in the regulation of appetite and body weight and showing a potential for the immunization therapy.

Conclusion

Autoantibodies directed against neuropeptides emerge as important participants of peptidergic transmission in physiologic and pathologic conditions. Due to changes in the variable region of immunoglobulin molecules, these autoAbs could be responsible for the variability in peptidergic signaling, ranging from transport to neutralization of the corresponding peptide messenger, as discussed in a recent review [38]. Our data suggest that in physiologic conditions this mechanism may participate in stress adaptation, whereas alterations may result in the development of eating and anxiety disorders. In fact, autoAbs directed against α-MSH appear as the primary pathogenic autoAbs in eating disorders, whereas the IgM class of α-MSH autoAbs is associated with increased satiety, and the IgG class of these autoAbs could trigger bulimic responses. Although the mechanism underlying alterations of α-MSH autoAb production leading to development of eating disorders has not yet been identified, the gut microflora appears as a putative target (Fig. 6). Conversely, neuropeptide autoAbs could also contribute to gastrointestinal disturbances frequently observed in patients with eating disorders [39] or after bariatric surgery [40] because several neuropeptides participate in the regulation of gastrointestinal functions (see reference by H. Cox [41]). Thus, based on the known pathogenesis of eating disorders, it would be possible to develop specific biological diagnostics and novel therapeutic approaches.

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