

Autoantibodies against neuropeptides are associated with psychological traits in eating disorders

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Previously, we identified that a majority of patients with anorexia nervosa (AN) and bulimia nervosa (BN) as well as some control subjects display autoantibodies (autoAbs) reacting with α -melanocyte-stimulating hormone (α -MSH) or adrenocorticotrophic hormone, melanocortin peptides involved in appetite control and the stress response. In this work, we studied the relevance of such autoAbs to AN and BN. In addition to previously identified neuropeptide autoAbs, the current study revealed the presence of autoAbs reacting with oxytocin (OT) or vasopressin (VP) in both patients and controls. Analysis of serum levels of identified autoAbs showed an increase of IgM autoAbs against α -MSH, OT, and VP as well as of IgG autoAbs against VP in AN patients when compared with BN patients and controls. Further, we investigated whether levels of these autoAbs correlated with psychological traits characteristic for eating disorders. We found significantly altered correlations between α -MSH autoAb levels and the total Eating Disorder Inventory-2 score, as well as most of its subscale dimensions in AN and BN patients vs. controls. Remarkably, these correlations were opposite in AN vs. BN patients. In contrast, levels of autoAbs reacting with adrenocorticotrophic hormone, OT, or VP had only few altered correlations with the Eating Disorder Inventory-2 subscale dimensions in AN and BN patients. Thus, our data reveal that core psychobehavioral abnormalities characteristic for eating disorders correlate with the levels of autoAbs against α -MSH, suggesting that AN and BN may be associated with autoAb-mediated dysfunctions of primarily the melanocortin system.

anorexia | autoimmunity | behavior | bulimia | hypothalamus

Neuropeptides are important transmitters in the brain limbic and neuroendocrine systems involved in the regulation of different modalities of feeding, social and defensive behaviors, the stress response, and homeostasis (1). These roles of neuropeptides suggest a possible implication in some neuropsychiatric conditions primarily manifested by behavioral abnormalities (2, 3), including anorexia nervosa (AN) and bulimia nervosa (BN) (4). Although changes in concentrations of some neuropeptides in plasma or in cerebrospinal fluid have been noted in patients with eating disorders (5, 6), the mechanisms underlying these changes are unknown, and the modern therapy of eating disorders adopts the symptomatic approach (7).

In search of possible peptidergic mechanisms underlying eating disorders, we recently found that a majority of a group of Swedish AN and BN patients display autoAbs against α -melanocyte-stimulating hormone (α -MSH) (8), a melanocortin peptide involved in appetite control (9–12). AutoAbs against adrenocorticotrophic hormone (ACTH) and luteinizing hormone-releasing hormone (LHRH) also were identified in some patients and controls (8). The growing evidence of the immune system's critical involvement in some neurological and psychiatric disorders (13) suggests that autoAbs reacting with neuropeptides responsible for the central control of appetite may partake in the pathogenesis of eating disorders.

In the present work, we further studied the occurrence of these autoAbs in AN and BN using a patient sample from Estonia. In these sera we in addition identified autoAbs reacting with oxytocin (OT) and vasopressin (VP), two neurohormones primarily involved in the regulation of water balance as well as in several motivated behaviors, including central mechanisms of social interactions (14, 15). Furthermore, to clarify the relevance of identified autoAbs reacting with neuropeptides to the symptoms of eating disorders, we tested our hypothesis that the levels of such autoAbs may correlate with the range of psychological problems in AN and BN patients. For this purpose, the widely used and reliable Eating Disorder Inventory (EDI-2) test (16, 17) was used to assess the cognitive and behavioral characteristics commonly found in individuals with AN and BN, while autoAbs levels were measured by ELISA.

Methods

Human Subjects. Sera from Estonian female patients with AN [mean age 19.5 yr, $n = 12$, body mass index (BMI) \pm SD, 16.3 ± 1.99] or with BN (mean age 21.5 yr, $n = 42$, BMI 20.8 ± 1.97) were used in this study. AN and BN were diagnosed by a psychiatrist and a clinical psychologist according to the 4th Ed. of *Diagnostic and Statistical Manual of Mental Disorders* (18). Sera from female volunteers (mean age 21.4 yr, $n = 41$, BMI 20.2 ± 2.41) served as the control group. Serum samples from AN and BN patients and controls were taken on the day of completing the EDI-2 test, instantly frozen, and kept at -70°C until processed for immunohistochemistry or ELISA.

Immunohistochemical Screening of Human Sera. To detect presence of IgG autoAbs against hypothalamic neuropeptides, sera from patients and controls were applied on rat hypothalamic and pituitary sections and processed immunohistochemically (8). Experiments were designed in accordance with guidelines on animal care and approved by the local ethical committee, Stockholms norra djurförsöksetiska nämnd. One day before they were killed, some rats were treated by intracerebroventricular injection of colchicine ($120 \mu\text{g}$ in $20 \mu\text{l}$ of 0.9% NaCl) to cause accumulation of neuropeptides in the cell soma due to interruption of axonal transport. To test the specificity of autoAb binding, the targeted molecules were identified by preadsorption of human sera with synthetic neuropeptides, as described in ref. 8 (for details see *Supporting Methods*, which is published as supporting information on the PNAS web site).

Abbreviations: α -MSH, α -melanocyte-stimulating hormone; ACTH, adrenocorticotrophic hormone; AN, anorexia nervosa; BMI, body mass index; BN, bulimia nervosa; EDI-2, Eating Disorder Inventory-2; LHRH, luteinizing hormone-releasing hormone; OT, oxytocin; VP, vasopressin.

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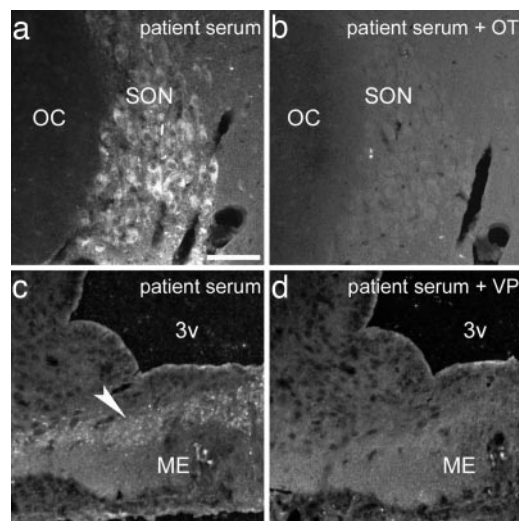


Fig. 2. Absorption of a patient serum with either synthetic OT (*b*) or VP (*d*) resulted in abolishment of OT/VP-like immunostaining in the rat supraoptic nucleus (SON, *b* vs. *a*) or the median eminence (ME, *d* vs. *c*). OC—optic chiasm, 3v—3rd brain ventricle. Arrowhead points to OT/VP-like immunopositive fibers in the ME. (Scale bar: 100 μ m.)

Levels of AutoAbs by ELISA. We used ELISA to measure levels of serum IgG autoAbs as well as to verify the presence and measure levels of IgM autoAbs reacting with α -MSH, ACTH, OT, or VP in patients and controls. The true Ab nature of autoAbs binding to synthetic peptides in microplates was proven, because F(ab)₂ fragment of IgG retained 75% of the binding shown by IgG. The binding was inhibitable in all four assay systems in a dose-dependent manner by competition with antigen. Large variations in the levels of autoAbs against all four neuropeptides were found in both patients and controls. Because of a nonhomogenous distribution of OD values within the groups as assessed by the Cochran-C test, a nonparametric statistical analysis was used to test for possible differences between the groups. Significantly increased (Kruskal–Wallis, $P < 0.01$) levels of IgM autoAbs against α -MSH, OT, and VP, as well as IgG autoAbs against VP were found in AN patients vs. either controls or BN patients, whereas IgG autoAbs levels against OT were found to be lower (Fig. 4).

Correspondence Between Immunohistochemical and ELISA Data. To analyze whether the immunohistochemical detection of autoAbs corresponded to the levels of autoAbs as measured by ELISA, the intensity of the neuropeptide-like staining was subjectively rated from 0 (no staining) to 3 (very strong). It appeared that only in the AN group the intensity of α -MSH-like staining correlated positively with the ELISA values for α -MSH autoAbs ($r = 0.76$, $P < 0.01$), whereas no such correlations were found in other groups or for other neuropeptide autoAbs. Accordingly, in

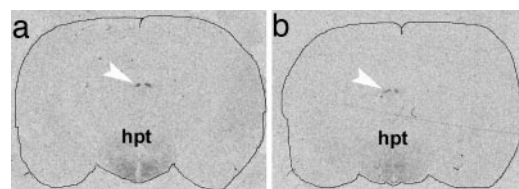


Fig. 3. Autoradiographs of ¹²⁵I-labeled α -MSH binding to the rat brain sections. (*a*) Control. (*b*) Preincubation with IgG, purified from the serum of an AN patient with a high α -MSH IgG level, significantly reduced signal intensity. Hpt, hypothalamus; arrowhead, medial habenula.

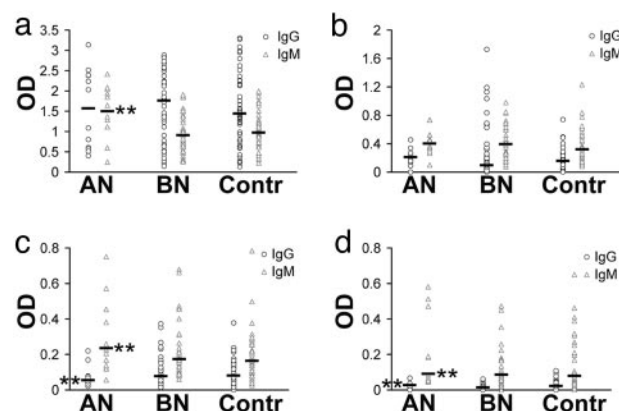


Fig. 4. Levels (OD, optic density in ELISA) of IgM and IgG autoAbs against α -MSH (*a*), ACTH (*b*), OT (*c*), and VP (*d*) in AN and BN patients and controls. Bars show median values (Kruskal–Wallis, **, $P < 0.001$ vs. controls).

the three AN sera that did not show α -MSH-like immunostaining, the OD values for α -MSH IgG levels were in the lowest range (0.3–0.8). Surprisingly, in the control sera, a similar low range of OD often was accompanied by visible α -MSH-like staining, whereas higher levels of OD did not correspond to the strong α -MSH-like staining. However, in our routine immunohistochemical studies, we also have sometimes experienced lack of correlation between titer and stainability. This phenomenon may be due to several factors, for example that not all Abs may react equally well on formalin-fixed tissue.

BMI and Neuropeptide AutoAbs. The BMI was significantly different among the patients and controls (ANOVA, $P < 0.01$). The BMI in AN patients was lower vs. controls and vs. BN patients (Fisher's least significant difference, mean BMI \pm SD, 16.3 ± 1.99 vs. 20.2 ± 2.41 and vs. 20.8 ± 1.97 , respectively; $P < 0.01$ for both), but the BMI of BN patients and of controls was not significantly different. Among all possible combinations evaluated between the BMI and neuropeptide Ig levels, significant Pearson's correlations were found between the BMI and α -MSH IgG levels in BN ($r = -0.46$, $P < 0.01$) and between the BMI and VP IgG levels in AN ($r = -0.6$, $P < 0.05$).

EDI-2 and Neuropeptide AutoAbs. The total EDI-2 scores differed significantly among the three groups (ANOVA, $P < 0.01$): AN patients (mean EDI-2 score \pm SD, 71.2 ± 40.8), BN patients (94.6 ± 42.2), and controls (34.7 ± 23.5). The total EDI-2 score was not significantly different between AN and BN patients, but both patient groups scored higher than controls (Fisher's least significant difference, $P < 0.01$ for both).

Screening of all of the combinations between the subscale score of the EDI-2 and the Abs levels as measured by ELISA, using our statistical model, we found several pairs that significantly differed among the controls and AN and BN patients. Most of the significant differences between patients and controls (β -correlations) were found in various psychological dimensions of EDI-2 vs. levels of autoAbs against α -MSH (Table 1). These differences were also significant for the total EDI-2 score (Table 1 and Fig. 5). Some significant β -correlations also were noted for autoAbs against ACTH, OT, and VP; such as a strong link between the levels of IgG autoAbs against ACTH and the score for Maturity Fears (AN, $\beta = 1.01$; BN, $\beta = -1.15$, $P < 0.01$); and a less strong link between levels of OT and VP IgM autoAbs and score for Bulimia (AN, OT, $\beta = -0.37$; VP, $\beta = -0.33$, $P < 0.05$ for both; BN, OT, $\beta = 0.37$, $P < 0.05$, VP, $\beta = 0.42$, $P < 0.01$). Remarkably, all of the β -correlations in AN were opposite from those in BN. To further analyze the origin of these differences,

Table 1. The β -correlations (AN and BN vs. controls) for pairs that showed significant differences among patients and controls

EDI-2 subscale	AutoAbs	
	α -MSH IgG (β)	α -MSH IgM (β)
Drive for thinness		
AN	0.70**	-0.64*
BN	-0.76**	0.51*
Bulimia		
AN	0.54*	-0.57*
BN	-0.43*	0.65**
Ineffectiveness		
AN	0.62*	n.s.
BN	-0.53**	n.s.
Interpersonal distrust		
AN	n.s.	-0.59*
BN	n.s.	0.85**
Ascetism		
AN	0.62*	n.s.
BN	-0.71*	n.s.
Impulse regulation		
AN	0.73**	-0.64*
BN	-0.5 n.s.	0.66*
Social insecurity		
AN	0.64*	n.s.
BN	-0.64*	n.s.
Total EDI-2		
AN	0.67**	-0.60*
BN	-0.58*	0.60*

β -correlations are for AN and BN vs. controls. Pairs are EDI-2 score/ α -MSH autoAbs level. *, $P < 0.05$; **, $P < 0.01$; n.s., not significant.

we searched for the correlation factors (Pearson's r) between Abs levels and EDI-2 total and subscale score. No significant Pearson's correlations for these pairs existed among controls,

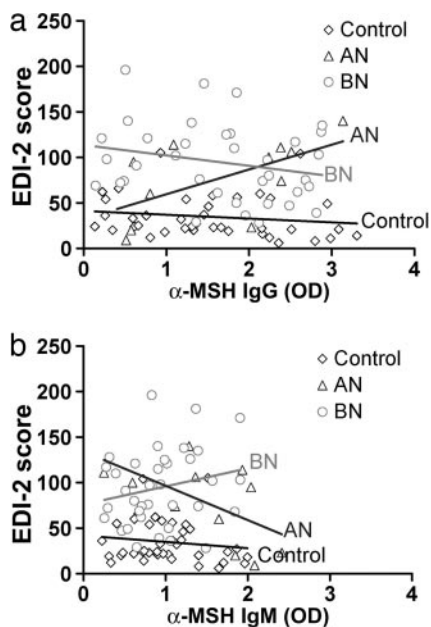


Fig. 5. Linear trend lines between the total EDI-2 score and levels of α -MSH IgG autoAbs (a) or α -MSH IgM autoAbs (b) in AN and BN patients and control subjects. The angles between the trend lines of the control group and those of AN or BN patients reflect β -correlations of the total EDI-2 score (Table 1). Note the opposite directions of the trend lines between the AN and BN groups as well as between IgG and IgM autoAbs.

Table 2. The correlation coefficients, Pearson's r , for pairs that showed significant β -correlations among AN and BN patients and controls

EDI-2 subscale/autoAbs	AN	BN	Contr
α -MSH IgG			
DT	0.55*	-0.36*	-0.01
B	0.45	-0.13	-0.14
IE	0.51*	-0.19	-0.22
A	0.61*	-0.28*	-0.04
IR	0.59*	-0.11	-0.17
SI	0.46	-0.26	-0.10
α -MSH IgM			
DT	-0.66*	0.08	-0.12
B	-0.41	0.3*	-0.01
ID	-0.55*	0.3*	-0.19
IR	-0.54*	0.18	-0.11
ACTH IgG			
MF	0.69**	-0.29*	-0.01
OT IgM			
B	-0.40	0.32*	0.23
VP IgM			
B	-0.26	0.43**	0.22
IA	0.17	0.45**	0.09

The correlation coefficients, Pearson's r (*, $P < 0.05$; **, $P < 0.01$) for those pairs (EDI-2 score/autoAbs level), that showed significant β -correlations among AN and BN patients and controls (Contr). A, Ascetism; DT, Drive for Thinness; B, Bulimia; BD, Body Dissatisfaction; IE, Ineffectiveness; IR, Impulse Regulation; P, Perfectionism; ID, Interpersonal Distrust; IA, Interoceptive Awareness; MF, Maturity Fears; and SI, Social Insecurity.

but many of them were present in AN and BN patients (Table 2). In contrast, for the pairs without significant β -correlations, some significant Pearson's correlations were found in all groups (Table 3).

Discussion

Possible Physiological Significance of Neuropeptide AutoAbs. Our work shows that autoAbs (IgG and IgM) against α -MSH, ACTH, OT, and VP can be detected in patients with eating disorders as well as in control subjects. The occurrence of neuropeptide autoAbs in control subjects suggests that autoAbs reacting with these neuropeptides/neurohormones might constitute a neuro-immunoendocrine mechanism that could be beneficial for the homeostatic control. The postulated functional role of neuropeptide autoAbs generally corresponds to the concept of chemical homeostasis proposed for natural autoAbs reacting with small molecules including some neurotransmitters and cytokines (23–26). Igs are relatively long-lived molecules compared with neuropeptides, i.e., the *in vivo* plasma half-lives for IgM and IgG are 5 and 23 days, respectively (27) vs. minutes for neuropeptides (e.g., 8 min for ACTH and 15 min for VP) (28). In this way, in normal conditions, autoAbs could serve as long-term regulatory factors, similar to well-known hormone-binding proteins, stabilizing/protecting and maintaining the levels of secreted neuropeptides/neurohormones.

Relevance of Neuropeptide AutoAbs to AN and BN. Our findings that the levels of IgM or IgG subclasses of autoAbs reacting with α -MSH, OT, or VP significantly differ between AN and BN patients or controls suggest that these autoAbs could be relevant to the development of eating disorders. However, because some control subjects displayed higher levels of autoAbs than some AN and BN patients, it is clear that their merely elevated serum levels are not sufficient to cause eating disorders. Nevertheless, our data revealing altered correlations between autoAbs levels and the psychological dimensions of the EDI-2 in AN and BN

group induced by a variety of the normal gut flora (47). Indeed, if the intestinal route is the main source of autoAbs reacting with neuropeptides, it might explain the higher incidence (63%) of α -MSH-like staining by sera from Estonian controls in the present study vs. 16% in previously studied Swedish controls (8), because significant differences in intestinal microflora have been found between Swedish and Estonian populations (48). The incidence of *H. pylori*, for instance, was reported to be as high as 56% in Estonian children ages 9–15 years (49) vs. only 10% in Swedish children of the same age (50). Certainly, a direct comparison of neuropeptide autoAbs levels with relation to gut microflora would be interesting.

Conclusion

In conclusion, we found that α -MSH autoAbs are associated with core psychobehavioral abnormalities, whereas autoAbs reacting with ACTH, OT, or VP are associated with fewer psychopathological traits characteristic for patients with eating disorders. Our

work provides previously undescribed evidence for a role of autoAbs reacting with hypothalamic neuropeptides in the origin of neuropsychiatric conditions characterized by distinct psychobehavioral abnormalities such as eating disorders. Based on our data and the known role of hypothalamic neuropeptides, we propose that autoAb-mediated dysfunctions of primarily the melanocortin system may contribute to the development of AN and BN. Further investigation of this previously undescribed concept in animal models could clarify the role of neuropeptide autoAbs in pathophysiology of AN and BN, with the aim of developing novel treatment strategies of eating disorders.

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