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Plasma agouti-related protein levels in women with anorexia nervosa

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Nutritional marker

Summary Agouti-related protein (AGRP) is the competitive antagonist of alpha-melanocyte stimulating hormone (α-MSH) located at melanocortin receptors 3 and 4 (MC3R and MC4R), and also acts as an MC4R inverse agonist. Hypothalamic AGRP controls food intake and body weight in rodents. It has also been found in human plasma. To study the possibility of disturbances in melanocortin receptor-related peptides in eating disorders, plasma AGRP, α-MSH, and leptin levels were measured in 18 female patients with anorexia nervosa (AN) (age, 23.5 ± 7.1 yr; body mass index (BMI) $14.5 \pm 1.8\,kg/m^2$) and 17 age-matched female controls (age, 25.8 ± 3.9 yr; BMI $20.2 \pm 1.6\,kg/m^2$). Blood samples were collected after overnight fasting, and plasma peptides levels were measured using ELISA. Plasma AGRP levels increased significantly in AN patients when compared with controls ($P<0.01$) while plasma α-MSH levels were not significantly different. Plasma leptin levels decreased significantly in AN patients when compared with controls ($P<0.001$). In addition, plasma AGRP levels were negatively correlated with leptin ($r = -0.41$, $P<0.01$) and BMI ($r = -0.40$, $P<0.05$) in all subjects. In conclusion, plasma AGRP elevation may be related to energy homeostasis disturbance in AN, and in addition to leptin, peripheral AGRP levels could be used as a nutritional marker in AN patients.

1. Introduction

Anorexia nervosa (AN) is an eating disorder characterized by decreased caloric intake, low weight, and reduced body fat. AN is diagnosed by weight loss and a refusal to maintain a minimal normal

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body weight, an intense fear of gaining weight or becoming fat, a self-evaluation unduly influenced by body shape and weight, and amenorrhea. Though the pathophysiology of AN is still unclear, an imbalance in neuropeptides controlling food intake and body weight has been found to be related to this disorder (Bailer and Kaye, 2003).

The hypothalamic agouti-related protein (AGRP) controls food intake and body weight in rodents (Schwartz et al., 2000). AGRP is the competitive antagonist of alpha-melanocyte stimulating hormone (α-MSH) located at melanocortin receptors 3 and 4 (MC3R and MC4R) (Schwartz et al., 2000), and also acts as an MC4R inverse agonist (Nijenhuis et al., 2001). AGRP mRNA has been found in the brain, adrenal gland, lung, and testis of humans (Ollmann et al., 1997; Li et al., 2000). It has also been found in human plasma. The function of systemically circulating AGRP, however, remains unknown.

Previous studies have shown that plasma AGRP and α-MSH are associated with obesity in human populations (Katsuki et al., 2001; Hoggard et al., 2004). Peripheral AGRP levels in blood increase with fasting (Shen et al., 2002; Gavrila et al., 2005). Also, an association between AN and AGRP gene polymorphism (G760A) has been reported (Vink et al., 2001), suggesting that AGRP may be related to the pathogenesis of AN.

In this study, the plasma levels of AGRP, α-MSH, and leptin in AN and healthy age-matched women were measured in order to determine the possibility of disturbance in these three peptides in AN. This is the first study to examine plasma AGRP and α-MSH in AN patients in relation to leptin.

2. Subjects and methods

2.1. Study subjects

Eighteen female AN patients (age, 23.5 ± 7.1 yr (mean $\pm$ SD)), who met the diagnostic criteria of the diagnostic and statistical manual of mental disorders-fourth edition (DSM-IV, American Psychiatric Association, 1994), and 17 age-matched healthy female controls (age, 25.8 ± 3.9 yr) were the subjects in this study. Among the 18 AN patients, 10 were binge-eating/purging type, whereas the remaining eight were restricting type. No patients had a previous diagnosis of bulimia nervosa. Study cases included outpatients and inpatients of the University of Tokyo Hospital. The age at onset in the AN patients was 19.8 ± 5.7 yr, and the duration of illness was 3.7 ± 3.1 yr. Drugs administered for treatment were biofermin (3 g/day; $n = 2$) and zolpidem (10 mg/day; $n = 2$) in AN patients, which are known not to affect nutritional status or energy balance. Controls were recruited by advertisement in the local community and were paid for their participation.

2.2. Study protocol

Blood samples from all subjects were collected after overnight fasting. Anthropometric measurements including height, body weight, and body fat were measured on the same day when blood samples were obtained. Percentage body fat was measured by bioelectrical impedance analysis (TANITA, Tokyo, Japan) (Katsuki et al., 2001). The protocol was approved by the Institutional Ethics Committee of the University of Tokyo, and written informed consent was obtained from all subjects prior to the study.

2.3. Laboratory measurements

All blood samples were drawn into chilled tubes containing EDTA-2Na (1 mg/ml), centrifuged immediately at 4 °C, and plasma portions were stored at −70 °C before analysis. All samples were analyzed in duplicate and in one assay to minimize variability. Plasma AGRP concentrations were measured by an ELISA kit (R&D systems, Minneapolis, MN, USA), with sensitivity of 0.68 pg/ml and intra-assay variability of less than 5.5%. Plasma α-MSH concentrations were measured using an EIA kit (Phoenix Peptide, Belmont, CA, USA), with sensitivity of 0.1 ng/ml and intra-assay variability of less than 5.0%. Plasma leptin concentrations were measured by an ELISA kit (R&D systems, Minneapolis, MN, USA), with sensitivity of 7.8 pg/ml and intra-assay variability of less than 3.3%.

2.4. Statistical analysis

Data distributions were examined for normality and homogeneity of variance by the Kolmogorov–Smirnov test. Because there was significant deviation from normality only in leptin data, logarithmic transformation was performed on leptin data. An unpaired *t*-test was used to assess differences in the two group comparisons. A value of $P < 0.05$ was considered statistically significant. The correlations between values were estimated by Pearson's correlation test. All statistical calculations were performed using SPSS for Windows version 10.0 (SPSS, Chicago, IL, USA).