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Decrease of c-Fos Expression in Hippocampus of Anorexia (*anx/anx*) Mice

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Mice homozygous for the lethal autosomal recessive anorexia mutation (*anx*) present with premature death around postnatal day 22. The anorexia mutant mice also present phenotypes such as reduced body weight, decreased food intake, and abnormal behavior characteristics such as body tremors, hyperactivity, uncoordinated gait, and head weaving. In order to investigate the expression of c-Fos in the hippocampus of anorexia mutant mice, the immunohistochemistry was performed in this study. The anorexia mutant mice exhibited lower expression of c-Fos in the hippocampus regions than the control group. In the CA3 and dentate gyrus, the number of c-Fos-positive cells in anorexia mutant mice was noticeably lower than that in control mice. However, no significant difference was found in the number of c-Fos-positive cells in CA1 of the two groups. The result suggests that the phenotypic characteristics of anorexia mutant mice may be associated with the hippocampal deficits of c-Fos expression.

Anorexia nervosa is one of severe eating disorders, and the criteria for diagnosis of anorexia nervosa are the simultaneous presentation; loss of weight to less than 85% of ideal weight, intense fear of obesity, body weight disturbance, and amenorrhea (American Psychiatric Association, 1994). The epidemiological prevalence is approximately 0.5-2 per 1000 women with the highest among the 15-19 yr old women, and the mortality rate is about 5-15% (Hoek, 1991; Joergensen, 1992). Several studies have showed that genetic factors play a pivotal role in the pathogenesis of anorexia nervosa (Collier et al., 1997; Bruins-Slot et al., 1998). On the basis of family and twin studies, some reports have suggested the role of genetic factors in the liability to anorexia nervosa (Holland et al., 1988; Stern et al., 1992; Rutherfold et al., 1993).

The anorexia mutant mouse (*anx/anx*) is a genetically anoretic mouse whose traits appear spontaneously. The autosomal recessive lethal anorexia mutation may cause starvation in preweanling stage and death around postnatal day 22. These mice show the phenotypic characteristics such as retarded growth, emaciation, and some neurological abnormalities e.g. (body tremors, abnormal gait, hyperactivity, and headweaving) (Son et al., 1994; Jahng et al., 1998; Kim et al., 2001). It is well-known that hippocampus is a responsible region for learning and memory. Hippocampus also has roles in eating behavior (Bendotti et al., 1982; Currie et al., 1998).

Transcription factors are proteins that control the expression of genes and the master regulators of development and function. Recently, it has become clear that transcription factors play crucial and specific roles in the nervous system development and function, as well as in the adaptive responses of the nervous system to many different types of stimuli, and to pathological situations (Herdegen and Leah, 1998). c-Fos is one of inducible transcription factors, and c-*fos* is expressed as an immediate-early gene. c-Fos protein is reported to be involved in modulation of transcription rates of target genes such as glucocorticoid receptors (Kerppola et al., 1993) and thyroid hormone receptors (Zhang et al., 1991). c-Fos is a transcription factor that may play a role in sensory stimulation, neurodegeneration and apoptosis, long term potentiation, seizure, hypoxia-ischemia, and brain development (Herdegen and Leah, 1998). However,

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little is known about the roles of c-Fos in the hippocampus of anorexia mutant mice.

To investigate the expression of c-Fos in the hippocampus of anorexia mutant mice, immunohistochemistry for c-Fos was performed. Here we report that anorexia mutant mice showed decreased expression of c-Fos in the hippocampus as compared to that of the control mice.

Materials and Methods

Animals

Homozygous anorexia mice (*anx/anx*) were produced from heterozygous breeder pairs (B6C3Fe-a/a-anx A/+a) obtained from the Jackson Laboratory. The experiments were performed in accordance to the animal care guidelines of NIH and the Korean Academy of Medical Sciences. Mice were housed under controlled temperature (20±2℃) and lighting (07:00-19:00) conditions. Homozygotes could be distinguished from normal littermates by their weight difference. Inhibition of normal growth in anorexia mutant mice is reported to result not from digestive failure, but from consumption of less food than is necessary. Thus, anorexia mutant mice were identified by their reduced body weight, body tremor, abnormal gait, and mild hyperactivity (Son et al., 1994; Jahng et al., 1998; Kim et al., 2001). Weanling mice (18-22 days old) were sorted into two groups: anorexia (n = 5) and control (n = 5). The mice were weighed and anesthetized with sodium pentobarbital until completely unresponsive. The anesthetized mice were transcardially perfused with 0.05 M phosphate buffered saline (PBS), and then fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4. The brains were carefully removed and post-fixed in the same fixative overnight and soaked in 30% sucrose solution for 48 h. The tissue was cut into 40 μm coronal sections using a freezing microtome (Leica). The mouse brain was analyzed using the atlas by Franklin and Paxinos (1997).

Materials

Sodium pentobabital, paraformaldehyde, sucrose, sodium phosphate monobasic, sodium phosphate dibasic, Trizma base, Trizma HCl, Triton-X, bovine serum albumin (BSA), goat serum, and diaminobenzidine (DAB) were purchased from Sigma. Permount was obtained from Fisher Scientific. c-Fos antibody was purchased from a commercial supplier (Santa Cruz). Elite ABC Kit was obtained from Vector Laboratories.

Immunohistochemistry

Immunohistochemistry was performed using an Elite ABC Kit and free-floating method as previously described (Huh et al., 1997; Kim et al., 2001). In brief, sections were incubated for 15 min in 3% hydrogen peroxide to remove endogenous peroxidase activity. The sections were then washed with PBS and incubated with c-Fos antibody (rabbit polyclonal IgG), diluted 1:1000 in PBS containing 1% BSA and 0.05% sodium azide overnight. The sections were reacted with secondary biotinylated goat anti-rabbit IgG (1:200) for 2 h, and avidin-biotin-peroxidase complex (ABC) for 1 h (1:100) at room temperature. For visualization of antibody reactivity, DAB as a chromogen was treated in the wells containing sections for 5 min. Finally, the stained section was mounted with Permount on a gelatinized glass slide.

Statistical analyses

Statistical analyses were performed using the statistical Package for the Social Sciences software SAS (version 6.1.2). Statistical differences were determined using Student's *t*-test, and $P < 0.05$ was considered to indicate statistical significance.

Results and Discussion

Body weight of anorexia mutant mice (average 3.7 g) was less than 50% of those of control mice (8.9 g) (data not shown).

In Fig. 1, c-Fos immunoreactivity was expressed highly in the dentate gyrus, moderately in CA1 and CA3 of both control and anorexia mutant mice. In control mice, c-Fos-positive cells of the dentate gyrus were strongly expressed in the granular layer; such cells were less frequently observed in the dentate gyrus of anorexia mutant mice. However, moderately expressed cells were scattered in the hilus of control mice, while less cells were observed in the mutant. The control mice showed moderate c-Fos expression in CA3 and no expression in CA2. The anorexia mice showed similar anatomical distribution but the number of c-Fos-positive cells in CA3 was less than that of the control mice. In CA1 of both control and anorexia mutant mice, the numbers of c-Fos-positive cells were similar.

Fig. 2 shows that the number of the c-Fos-positive cells in the hippocampus of anorexia mutant mice are significantly lower than that of the control mice. The number of c-Fos-positive cells per section was 80.61±5.59 (mean±standard error) in the dentate gyrus of the control mice. The number of dentate c-Fos-positive cells (52.78±3.51) in the anorexia mice was significantly lower. The numbers of c-Fos-positive cells in CA1 and CA3 of control mice were 364.08±19.73 and 86.14±4.56. In the anorexia mice, the c-Fos-positive cells of CA3 (34.52±3.12) were significantly lower than that of the control mice, while that of CA1 (346.76±21.78) was only slightly lower.

Eating disorders represent a spectrum of overlapping conditions that combine disturbances in eating behavior. Estimates of the prevalence of eating disorders

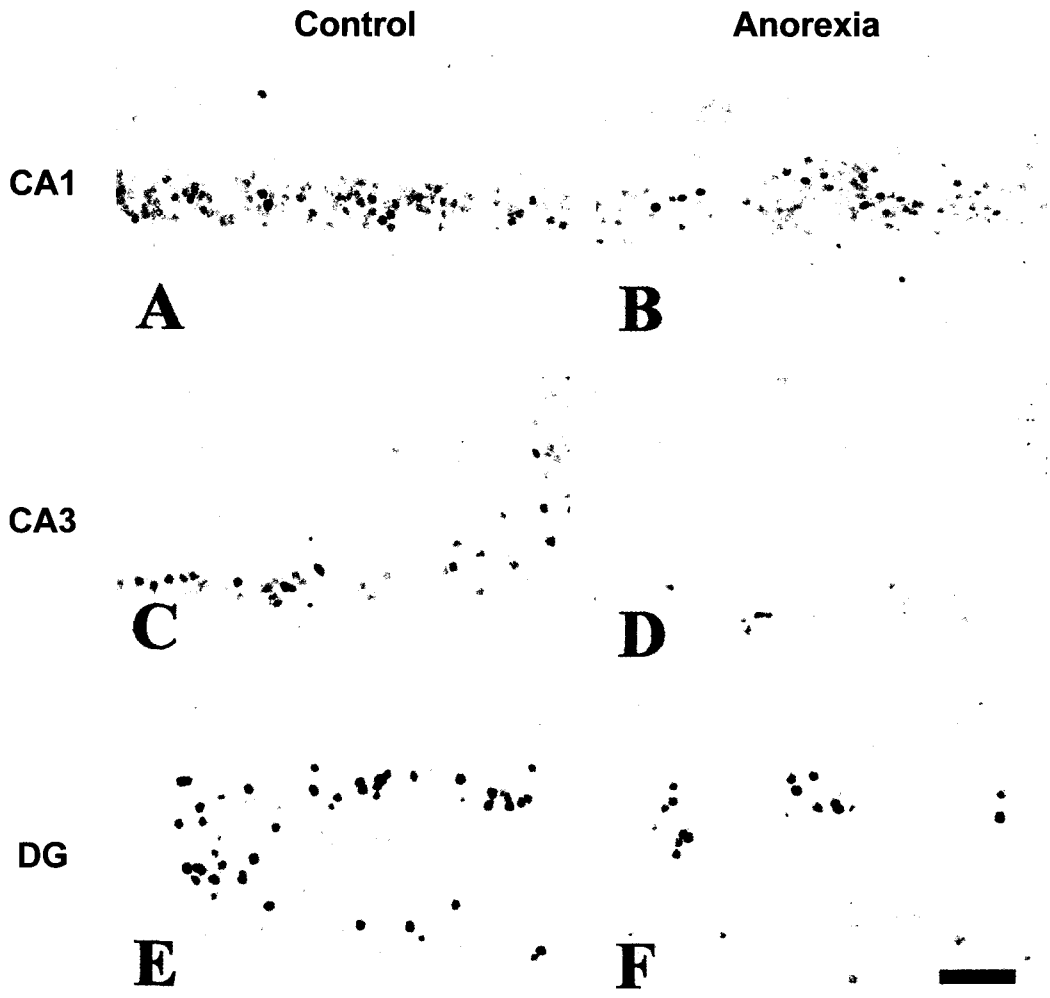

Fig. 1. Decrease in the c-Fos-positive cells in the hippocampus of anorexia mutant mice. Each section was stained for c-Fos (brown). CA1 (A, B); CA3 (C, D); DG (E, F), dentate gyrus; Control (n = 5); Anorexia (n = 5); Scale bar = 90 μm.

are less than 1% for anorexia nervosa, 1-3% for bulimia nervosa, and 2-5% for binge eating disorder. Among the eating disorders, anorexia nervosa has a high mortality rate, approximately 5-19% (American Psychiatric Association, 1994). The pathogenesis of anorexia nervosa may be related to a complex interplay of genetic, social, and psychologic factors. Anorexia nervosa patient shows distinct characteristics such as weight loss, fear of weight gain, disturbance of body image, and amenorrhea (Gorwood et al., 1998; Stoving et al., 1999). Despite the remarkable studies in eating behavior during past decades, little is known about the pathophysiology of anorexia nervosa. Moreover, the role of c-Fos in the pathogenesis of anorexia nervosa has not been studied yet.

The viral '*fos*' gene was isolated in 1982 as the oncogene of the Finkel-Biskis-Jinkins murine osteogenic sarcoma virus (Curran and Teich, 1982). Its cellular counterpart, c-*fos*, was found by Curran et al. (1983) and characterized as nuclear protein (Curran et al., 1984). DNA-binding by c-Fos depends mainly on the basic residues adjacent to the leucine zipper domain. c-Fos proteins cannot form homodimers, but dimerize with Jun proteins. c-Fos:Jun dimers attach to AP-1 and CRE sites. In rat, the most extensive Fos expression occurs at about postnatal day 14 (P14). At this time there are strong expressions in the olfactory bulb, the frontal, orbital, and cingulate areas of the frontal cortex, and in the septum; and moderate expressions in the pyriform and entorhinal cortices, CA1 and CA3, dorsal endopyriform nucleus, caudate, anteroventral thalamus, amygdala, and tegmentum (Jensen et al., 1993; Kasof et al., 1995). In mouse, c-Fos appears on P10 in a small number of cells in the hippocampus. On P30 it is present in hippocampal pyramidal cells and granule cells in the dentate gyrus (Herdgen and Leah, 1998). The anterior cingulate, CA3, raphe nuclei, ventral tegmentum, and central grey display low to

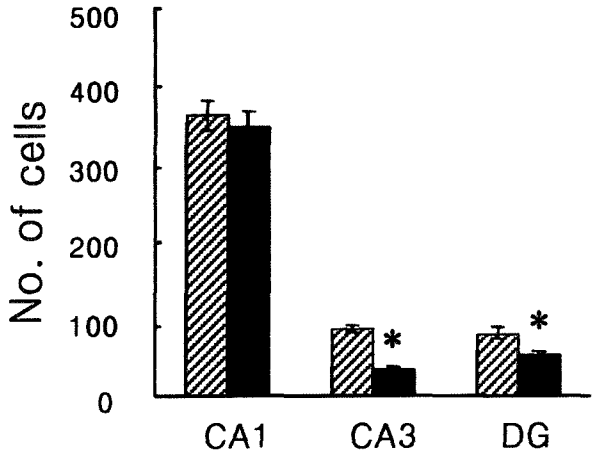

Fig. 2. Number of c-Fos-positive cells in the various hippocampal areas. Values are represented as mean±standard error (bar). DG, dentate gyrus; Control (▨, n = 5); Anorexia (■, n = 5). Statistically significant decrease ($P < 0.05$) compared to the number of c-Fos-positive cells of control mice.

moderate expression of c-*fos* throughout most of the postnatal time; whereas the nucleus accumbens, subthalamus, dentate gyrus, inferior colliculus, medial geniculate, and cerebellar Purkinje cells never display labelling (Smeyne et al., 1992). c-Fos has been known to be a marker of neuronal activation in the nervous system (Huang and Wang, 1998).

The anorexia mutant mice display an autosomal recessive trait and show decreased food intake and body weights compared to their wild-type littermates. Although the molecular mechanism of anorexia nervosa is unknown, this animal model will provide basic information on the anorexic phenomena.

In conclusion, this study showed that anorexia mutant mice have decreased c-Fos expression in the hippocampus. This implicates that the phenotypic characteristics of anorexia mutant mice may be associated with deficits of c-Fos expression in the hippocampus. Thus, we suggest that this phenomenon may, in part, involve in the molecular mechanism and/or neurological deficits of anorexia nervosa. The anorexia mutant mice will also be a useful animal model for the basic studies of eating disorders, particularly in exploring the molecular mechanism of anorexia nervosa.

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