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Dexamethasone reduces food intake, weight gain and the hypothalamic 5-HT concentration and increases plasma leptin in rats

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Abstract

This study was conducted to define the regulatory mechanisms underlying stress-induced decreases in food intake and weight gain. Rats received a single or 4 daily injections of dexamethasone (0.1 or 1 mg/kg). Food intake and weight gain were recorded, and plasma leptin, brain contents of serotonin (5-hydroxytryptamine; 5-HT), 5-hydroxy-indole-acetic acid (5-HIAA) and the raphe expression of tryptophan hydroxylase (TPH), monoamine oxidase A (MAO-A) and 5-HT reuptake transporter (5-HTT) genes were examined. A single injection of dexamethasone did not acutely affect food intake, but cumulative food intake and weight gain were suppressed dose-dependently by daily injections of dexamethasone. Both a single and repeated injections of dexamethasone elevated plasma leptin in a dose dependent manner. 5-HT contents in the hypothalamus was decreased, but 5-HIAA increased, both by a single and repeated dexamethasone. A single injection of dexamethasone did not affect mRNA expressions of TPH, MAO-A and 5-HTT genes, but repeated dexamethasone increased them in the dorsal raphe nucleus. These results suggest that plasma leptin may play a role in dexamethasone-induced anorexia. Additionally, increased expression of MAO-A and 5-HTT genes by repeated dexamethasone appears to be implicated in decreases of the brain 5-HT contents.

Keywords: Anorexia; Hypothalamus; Serotonin reuptake transporter; Stress

1. Introduction

Chronic or repeated stress, such as chronic unpredictable mild stress (Lin et al., 2005) and repeated exposure to restraint or immobilization (Harris et al., 2006; Makino et al., 1999), results in reduction of food intake and body weight gain in rats. Stressful stimuli cause glucocorticoid release by the adrenal glands (Axelrod and Reisine, 1984), and adrenal glucocorticoids have been implicated in the regulation of energy homeostasis (Cavagnini et al., 2000). In rodents, centrally administered glucocorticoids increase food intake and weight gain, but peripherally administered glucocorticoids suppress them (Zakrzewska et al., 1999). Taken together, it is suggested that adrenal glucocorticoids may mediate stress-induced anorexia; however, no clear link between increased plasma glucocorticoid concentration by stress and diminution in food intake and weight gain has been reported.

Serotonin (5-hydroxytryptamine; 5-HT) modulates food intake and energy expenditure (Le Feuvre et al., 1991; Meguid et al., 2000). Pharmacological activation of the central 5-HTergic system has been shown to exert anorexic effects, both experimentally and clinically (Nielsen et al., 1992; Ward et al., 1999). Food intake increases extracellular levels of 5-HT in the medial and the lateral hypothalamic regions (Mori et al., 1999; Schwartz et al., 1990), and plasma corticosterone is reported to be implicated in food-induced hypothalamic 5-HT release

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(Guimaraes et al., 2002). Changes in plasma corticosterone levels or exogenous glucocorticoids alter gene expression of 5-HT receptors in various brain regions in rodents (Holmes et al., 1995, 1997; Katagiri et al., 2001). Glucocorticoid receptors are expressed in 5-HT neurons (Fuxe et al., 1987), and adrenalectomy reduces and corticosterone replacement restores the rate of 5-HT synthesis (Dinan, 1996).

Plasma leptin level is known to regulate food intake; e.g., leptin administration suppresses feeding and increases energy expenditure resulting in body weight loss in rodents (Pelleymounter et al., 1995; Schwartz et al., 1996). Leptin is also considered to be a stress-related hormone and its secretion is stimulated by stress (Hernandez et al., 2000; Konishi et al., 2006; Wallace et al., 2000). Synthetic glucocorticoid dexamethasone increases leptin mRNA expression in the adipose tissue (Lee et al., 2007) and induces long-lasting hyperleptinemia in rats (Caldefie-Chezet et al., 2001).

This study was conducted to define the regulatory mechanisms underlying stress-induced decreases in food intake and weight gain. We hypothesized that stress-induced anorexia may involve alterations in plasma leptin level and brain 5-HTergic activity. Rats were treated with pharmacologic doses of dexamethasone, mimicking a stress response (Caldefie-Chezet et al., 2001, 2005), and plasma leptin, brain contents of 5-HT,5-hydroxy-indole-actic acid (5-HIAA) and the raphe expression of tryptophan hydroxylase (TPH), monoamine oxidase A (MAO-A) and 5-HT reuptake transporter (5-HTT) genes were examined.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (8 weeks of age) were purchased from Daehan biolink Co. (Korea), and cared in a specific-pathogen-free barrier area with constant control of temperature (22±1 °C), humidity (55%), and a 12/12 h light/dark cycle (lights-on at 07:00 AM). Standard laboratory food (Purina Rodent Chow, Purina Co., Seoul, Korea) and membrane filtered purified water were available *ad libitum*. Animals were cared according to the Guideline for Animal Experiments, 2000, edited by the Korean Academy of Medical Sciences, which is consistent with the NIH Guidelines for the Care and Use of Laboratory Animals, revised 1996.

2.2. Drug treatments

Dexamethasone (sodium dexamethasone, Sigma Co.) was dissolved in aseptic physiologic saline at a concentration of 5 mg/ml. Rats with 310–380 g of body weights received a single injection or 4 daily injections of dexamethasone intraperitoneally at doses of 0, 0.1, or 1.0 mg/kg. Rats were evenly distributed over the different treatment groups by their weights. Each rat received the same injection volume at 5:00 PM, and was sacrificed 2 h after a single injection; i.e., at 7:00 PM, or the next morning after the 4th injection (4 daily injections). Dexamethasone injection was given in the evening when the spontaneous feeding activity of laboratory rats is increased. Food intake and body weight gain were recorded, and the pair-fed group received the same amount of chow that was consumed by high dose dexamethasone (1.0 mg/kg) group.

2.3. 5-HT and 5-HIAA contents in each brain region

Rats ($n=6$, 18 rats with a single injection and 18 rats with 4 daily injections, total 36 rats) were rapidly decapitated after brief anesthesia in a carbon dioxide chamber. Tissue samples of the hypothalamus and the midbrain raphe were rapidly dissected on ice immediately after decapitation, frozen in liquid nitrogen and stored at −80 °C until used. 5-HT and 5-HIAA contents of the tissue samples were measured by high-performance liquid chromatography (Waters Instrument, Model 700, Milford, MA, USA) equipped with an ESA Coulochem II Electrochemical Detector (ESA Inc., Chelmsford, MA, USA) packed by biophase ODS 5 (mm (250×4.5 mm, Bioanalytical System Inc., West Lafayette, IN, USA) according to a modification of the method previously reported (Wagner et al., 1982). The mobile phase, comprising of 8% acetonitrile and 92% 0.15 M monochloroacetic acid buffer (0.55 mM sodium octyl sulfate, 2 mM disodium EDTA, pH 3.35) was pumped at a rate of 1 ml/min.

2.4. In situ hybridization

Another groups of rats ($n=6$, 18 rats with a single injection and 18 rats with 4 daily injections, total 36 rats) were anesthetized with an overdose of sodium pentobarbital. Once unresponsive, transcardiac perfusion was performed with heparinized isotonic saline (0.9% NaCl, 0.5% $NaNO_2$) followed by ice-cold fixative (4% paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.2). Brains were rapidly dissected, blocked, and post-fixed in the same fixative for 3 h, and then transferred into 30% sucrose solution for 24 h for cryoprotection. Forty-micron coronal sections were cut on a freezing sliding microtome (MICROM Laborgeräte, Walldorf, Germany). Every third sections of the raphe nucleus (between bregma −7.64 mm and −8.80 mm; Paxinos and Watson, 1986) were collected into 20 ml glass scintillation vials containing ice-cold 2×SSC (0.3 M NaCl, 0.03 M sodium citrate). The SSC was pipetted off, and the sections were suspended in 1 ml of prehybridization buffer (50% formamide, 10% dextran sulfate, 2×SSC, 1×Denhardt's solution, 50 mM dithiothreitol, and 0.5 mg/ml denatured herring sperm DNA), incubated for 2 h at 48 °C. *In situ* hybridization was performed with radioactively labeled cDNA probes of TPH (Park et al., 1993), MAO-A (Jahng et al., 1997), or 5-HTT (Jahng et al., 1998) as we previously described (Choi et al., 2003). The tissue sections were then mounted on gelatin-subbed slides, air-dried, and apposed to Kodak BioMax film (Eastman Kodak Co., NY, USA) at 4 °C. Exposure times varied from 12 to 48 h to obtain autoradiographic images within a linear range of optical density after development in Kodak D-19 developer. *In situ* hybridization was carried out on the representative members of each experimental group at the same time under identical conditions, allowing direct comparison of mRNA