Anorexia, anhedonia and depression in rats by chronic dexamethasone

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Abstract

This study investigated a causal relationship between anorexia and anhedonia in an animal model of stress, and examined if the stress-induced anorexia and/or anhedonia is accompanied by changes in sweet receptor expression in taste cells. Rats received daily injections of dexamethasone (0.1 or 1 mg/kg) or saline, mimicking stress status. Anhedonia was assessed by measuring preferences for sweet and palatable foods at different time points of drug injections. Rats were subjected to behavioral sessions to assess depression-like behaviors after 3 days or 4 weeks of daily drug injections. Decreased food intake was observed from the first day of dexamethasone injection in a dose dependent manner, and persisted throughout the whole experimental period, revealing anorectic property of the drug treatment. Sucrose preference was decreased in the 2nd week, and cookie consumption on the 4th day, of drug treatment. Increased immobility during forced swimming test was observed at the 3rd week, but not on the 4th day, of drug treatment. Sweet receptors, T1R2 and T1R3, expression in the circumvallate papillae was not affected by dexamethasone treatment. It is concluded that the development of anhedonia, possibly caused by anorexia, is a pre-symptomatic feature of depression in rats treated with chronic dexamethasone, and its pathophysiologic mechanisms may not comprise changes in sweet receptor expression in taste cells.

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Introduction

Gustatory system contributes to judge nutritious qualities and toxic profiles of food by decoding the chemical information of tastants (Verschoor et al., 2010), and evaluating the taste quality is highly related with cognitive and behavioral features of hedonic processing as well as aversive (Dotson et al., 2012; Dando et al., 2013). However the functional physiology of taste is known to be affected by various psychopathological factors including stress (Nakagawa et al., 1996; Boltong and Keast, 2012).

Prolonged or repeated exposure to stressful events has been associated with clinical depression in humans and depressive-like behaviors in rodent models (Chandrashekar et al., 2006; Jenkins et al., 2010). Anhedonia is a core symptom of depression, and the concept of anhedonia refers to a reduction of the ability to experience pleasure, as reflected in a diminished interest in rewarding stimuli and pleasurable events (Der-Avakian and Markou, 2012). A possible dysfunction in the reward and motivation systems has been lately proposed to explain the link between anhedonia and depression (Pizzagalli, 2014).

Several studies have reported on the anhedonic aspect of gustation in depression and stressed conditions (Swiecicki et al., 2009; Furay et al., 2011). Symptoms of anhedonia is frequently found in patients with anorexia (Keating et al., 2012). A decreased sweet sensation has been reported in patients with anorexia (Simon et al., 1993) and test subjects under stressful condition (Nakagawa et al., 1996; Al’Absi et al., 2012; Ileri-Gurel et al., 2013). In rodent models, anhedonia can be easily measured by decreased consumption of sweet foods. Repeated restraint stress suppressed the integrated response to sweet taste in rats, and this was accompanied by decreased expression of sweet receptor gene in the peripheral taste structure (Okamoto et al., 2010). Taken together, it is suggested that anhedonia may be associated with stress-induced anorexia, possibly comprising changes in sweet receptor expression in the taste sensing cells.

It has been reported that animal models of depression comprising anhedonia exhibit dysfunctions of the hypothalamic-pituitary-adrenal (HPA) axis (Torres and Nowson, 2007), and the HPA axis dysfunctions characterized by increased corticotropin-releasing hormone and cortisol levels are pointed for the main factor of anorexia (Licinio et al., 1996). Considering several animal models of depression have shown reduced food intake and weight gain accompanied with decreased sweet solution intake (Merali et al., 2003), it is suggested that anorexia and anhedonia may share the stress axis dysfunction in its pathophysiological mechanism. Studies have reported that daily treatment with pharmacological doses of dexamethasone, mimicking a stress state (Caldefie-Chezet et al., 2001, 2005), induces anorexia with reduced food intake and weight gain (Jahng et al., 2008) and anhedonia with reduced sweet preference (Casarotto and Andreatini, 2007; Sigwalt et al., 2011). In this study, rats were treated daily with pharmacological doses of dexamethasone, in order to investigate the pathophysiology of anhedonia in an animal model of stress-induced anorexia.

Materials and methods

Animals

Six-week-old male Sprague-Dawley rats weighing 200–250g were purchased (Orient Bio, South Korea), and cared in a specific-pathogen-free (SPF) barrier area with constant control of temperature (22±1°C), humidity (55%), and a 12/12 hr light/dark cycle (lights-on at 07:30 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. All animal experiments were approved by the Committee for the Care and Use of Laboratory Animals at Seoul National University.

Drug treatments

Dexamethasone (Dae Won INJ, Korea) was prepared freshly and dissolved in aseptic physiologic saline at a concentration of 5 mg/ml. Rats received
intraperitoneal injection of dexamethasone daily 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 during 9:00 AM - 9:30 AM.

Sweet preference tests
For sucrose preference test, rats were deprived from water, but not food, overnight (PM 19:00 to AM 9:00) before each test day. On test days, rats were subjected to a preference test on 1% sucrose solution to water for 30 min with two-bottle choice paradigm at 30 min after drug injection. Sucrose preference test was performed on day 2, 9, 16 and 23 of drug injections, and the positions of the sucrose and water bottles were counterbalanced across test days in each cage. The amounts of sucrose solution and water consumed during the 30 min of each test session were recorded, respectively. Preference scores of sucrose solution to water on each test day were calculated.

For cookie preference test, rats had free choices of chocolate cookies (Oreo cookie; Nabisco, East Hanover, NJ, USA) and chow at 30 min after the drug injection daily for 5 days. The amounts of cookies and chow consumed during 1 h of each test session were measured by weight daily.

Ambulatory activity
Rats were subjected to the ambulatory activity test after the 3rd or the 24th of daily drug injections. On each trial, the rat was placed in the center of the activity chamber (43.2 cm in length, 42.2 cm in width, and 30.5 cm in height, MED Associates, VT, USA), a transparent acryl chamber equipped with two horizontal planes of 16 infra red photocell-detector pairs placed in x, y dimension, spaced 2.5 cm apart, and its ambulatory activity was monitored by the computerized system for 60 min. Light condition of the test room was maintained in the same intensity with animal rooms under day-light condition. Ambulatory activity was measured as the total counts of beam interruptions in the horizontal sensor during each consecutive 5 min session. The activity chamber was cleaned with 70% ethanol after each use to eliminate any olfactory cues of the previously tested rat.

Forced swim test
On the next day of the ambulatory activity test, rats were subjected to the forced swim test, following the method previously described (Porsolt et al., 1977). Each rat was allowed to swim in a glass cylinder (54 cm in height and 24 cm in diameter) filled with water in 40 cm of depth (23-25°C) for 5 min, and the test sessions were recorded by a video camera from the side of the cylinder. Duration of rat’s immobility in the water was scored from videotapes by a trained observer who was blinded to the experimental conditions. Immobility was defined as the state in which rats were judged to be making only the movements necessary to keep their head above the surface.

Rats were placed in the test room at least 2 h prior to each test to minimize unwanted stress effects, and behavioral assessments were performed between 9:00 AM and 12:00 PM of the day to avoid the influences of circadian variances. The intraperitoneal injections of dexamethasone or saline vehicle were given 30 min before each test.

Taste bud RNA isolation
Tongue tissues were collected immediately after decapitation on the 4th or 28th day of drug injections. Tongue tissues containing the circumvallate papillae were rapidly dissected and placed in ice-cool Tyrode solution. Collagenase D cocktail solution (5mg/ml Collagenase, 24u/ml Dispase, 10mg/ml Tripsin inhibitor dissolved in Tyrode solution) was injected under the lingual epithelium layer, and the tissues were incubated at 37°C water bath for 40 min. The lingual epithelium was peeled off and taste buds were isolated by gentle agitation. The total RNA of isolated taste buds was extracted by Isol-RNA Lysis Reagent (5Prime#2302700) according to manufacturer’s instructions.

Quantitative real-time PCR
Quantitative real-time PCR was done with Power
SYBR Green PCR master mix (Life technologies #4367656). cDNA was synthesized with Super Script II Reverse Transcriptase (Invitrogen #18064) with 1μg of taste bud RNA. The primers were used in table1 with cycle of stage1; 94℃/5min, stage2; 94℃/ 45sec, 61℃/ 45sec, 72℃/ 45sec and satge3; 95℃/60sec, 60℃/1min, 95℃/15sec, 60℃/ 15sec. 40 reaction cycles was repeated at stage2 and data was collected at stage3, using a 7300 Gene Amp PCR system (Applied Biosystems). All data were normalized by standard for results.

Statistical Analysis
All data were analyzed by one-way analysis of variance (ANOVA) and preplanned comparisons between groups performed by post hoc Fisher’s PLSD test, using StatView software 5.0 (Abacus, Berkeley, CA, USA). Sweet preference tests were further analyzed by repeated measures ANOVA. Significance was set at P<0.05 and all values were presented as means ± S.E.M.

Results
Body weight and food intake
Body weight gain was suppressed in a dose dependent manner, and the dose effect of drug injections was firstly found on day 3 (P<0.05, Saline vs. Dexa 0.1 or D saxa 0.1 vs. D saxa 1.0) (Fig. 1A). Dexamethasone significantly suppressed food intake, and its dose effect on food intake was observed during the first week (P<0.05, Saline vs. D saxa 0.1 or D saxa 1.0 on each day, except day 4), but not the second and third week, of daily dexamethasone (Fig. 1B).

Sweet preference tests
Preference scores on 1 % sucrose solution were reduced in the high dose (1.0 mg/kg) group (P<0.05), but not in the low dose (0.1 mg/kg) group, in the 2nd and 3rd week of dexamethasone treatment, as compared to the saline control group (Fig. 2A). Analysis of sucrose preference with repeated measures ANOVA revealed main effect of treatment [F(2.20) = 7.287, P = 0.0042] during days 16 and 23, and main effect of day [F(2.60) = 4.421, P = 0.0071] during days 2 and 23. Cookie consumption was significantly reduced both in the high dose and the low dose groups on the 4th day (P<0.05 vs. saline, respectively), and only in high dose group on the 5th day, of drug treatment (Fig. 2B). Repeated measures ANOVA revealed main effect of treatment [F(2.14) = 4.671, P = 0.0279] on cookie intake during the 4th and 5th days of drug treatment. Chow intake during the test session did not differ among all groups.

Fig. 1. Body weight gain (A) and daily food intake (B). Rats received an intraperitoneal dexamethasone (0.1 or 1.0 mg/kg) or saline with the same injection volumes daily at 09:00 AM. D saxa 0.1; dexamethasone 0.1 mg/kg, D saxa 1.0; dexamethasone 1 mg/kg, *P<0.05 vs. Saline, *P<0.05 vs. D saxa 0.1, n=6 in each group, Data are presented by means ± S.E.M.

Behavioral assessments
Rats were subjected to the ambulatory activity test on day 3 and 24 of daily drug injections (Fig. 3). Ambulatory counts were not affected by dexamethasone treatment at both time points. However, center zone activity during the ambulation test; i.e. central distance traveled/total distance
traveled, was significantly decreased in the high dose group on day 24 ($P<0.05$), but not on day 3, compared to the saline control group. Rats were subjected to the forced swim test on the next day of the ambulatory activity test (Fig. 4). Immobility duration during the swim test was significantly increased in the high dose dexamethasone group on day 25 ($P<0.05$), but not on day 4, compared to the saline control group. Immobility of the low dose group tended to be increased on day 25 compared with the saline control group without statistical significance.

**Fig. 2.** Sweet preference tests. For sucrose preference test, rats were deprived from water, but not food, overnight before each test day, and then subjected to a preference test on 1% sucrose solution to water for 30 min with two-bottle choice paradigm at 30 min after drug injection (A). For cookie preference test, rats had free choices of Oreo cookies and chow for 1 h after the drug injection with a 30 min interval (B). Dexa 0.1; dexamethasone 0.1 mg/kg, Dexa 1.0; dexamethasone 1 mg/kg, *$P<0.05$ in (A), *$P<0.05$ vs. Dexa 0.1 or 1.0, *$P<0.05$ vs. Dexa 1.0, n=6 in each group, Data are presented by means ± S.E.M.

**Sweet receptors expression**

Gene expression of sweet receptors T1R2 and T1R3 in the taste cells of circumvallate papillae was examined with Q-PCR after 4 days or 4 weeks of daily drug injections. As shown in Fig. 5, daily dexamethasone did not affect the circumvallate expression of T1R2 and T1R3 at both time points examined.

**Fig. 3.** Ambulatory counts and the center zone activity during 60 min of ambulatory activity test performed on the 3rd and 24th day of drug treatment, respectively. The center zone activity was calculated by center zone travel distance/total travel distance. Dexa 0.1; dexamethasone 0.1 mg/kg, Dexa 1.0; dexamethasone 1 mg/kg, *$P<0.05$ vs. Saline, n=6 in each group, Data are presented by means ± S.E.M.

**Discussion**

Decreased food intake and weight loss has been suggested to serve as the most reliable marker of stress severity (Armario, 2006). In adult rats, repeated exposure to restraint or immobilization results in reduction of food intake and body weight gain (Makino et al., 1999; Harris et al., 2006). Adrenal glucocorticoids have been suggested to mediate stress-induced anorexia; i.e. stressful stimuli cause glucocorticoid release by the adrenal glands (Axelrod and Reisine, 1984); adrenal glucocorticoids have been implicated in the regulation of energy homeostasis (Cavagnini et al., 2000); peripherally administered glucocorticoids suppress food intake and weight gain in rodents (Zakrzewska et al., 1999). Present study demonstrated that daily injections of
synthetic glucocorticoid dexamethasone, mimicking stress condition, suppress both food intake and weight gain in a dose dependent manner and the dexamethasone-induced anorexia is observed from the first day of drug treatment, further supporting the idea that adrenal glucocorticoids may mediate the stress-induced anorexia in accordance with the previous report (Jahng et al., 2008). It has been reported that consumption of sucrose solution or sweet food is progressively reduced in rats by chronic mild stress, revealing stress-induced anhedonia (Willer et al., 1996; Lin et al., 2005; Luca et al., 2008; Tacchi et al., 2008). Rats treated acutely with dexamethasone at 5-10 mg/kg doses showed anhedonia; i.e. a significant decrease in sucrose preference in comparison to vehicle treated rats, although 1 mg/kg dexamethasone did not acutely alter the sucrose preference (Casarotto and Andreatini, 2007). In this study, it is demonstrated that chronic dexamethasone suppresses the sucrose preference after 2 weeks of drug treatment at 1 mg/kg dose in accordance with the case of chronic mild stress (Willer et al., 1996); however, it suppresses cookie consumption by 4 days of drug treatment even at lower (0.1 mg/kg) dose. A recent study have reported that anhedonic feature of daily dexamethasone at 1.5 mg/kg dose which measured by sucrose preference test is observed after 3 weeks of drug treatment, but not before (Sigwalt et al., 2011). It is concluded that glucocorticoids may play a key role in the development of anhedonia associated with stress-induced anorexia, and suggested that cookie preference test can be used as a better index than sucrose preference test for earlier detection of stress-induced anhedonia.

Symptoms of anhedonia is frequently found in patients with anorexia (Keating et al., 2012) and anhedonia in the animal model of chronic mild stress appeared to be associated with anorexia; i.e. reductions in food intake and body weight gain per se (Lin et al., 2005; Luca et al., 2008). However, the causal relation between anhedonia and anorexia is not clear yet. In this study, dexamethasone-induced anorexia, reduced food intake and weight gain, was observed on the first day, but anhedonia on the 4th day, of drug treatment at the earliest time point, suggesting that anorexia can be a cause to induce the development of anhedonia. It has been reported that anhedonia may be a pre-symptomatic feature in the patients with depression (Pizzagalli, 2014). In this study, anhedonic property of dexamethasone was observed with the cookie test on the 4th day of drug treatment; however, the drug-induced depression-like behaviors during forced swimming test was not observed on the 4th day, but it was on the 4th week. Thus, it is suggested that anhedonia may be a pre-symptomatic feature of depression in the animal model of stress-induced anorexia.

It was reported that chronic restraint stress suppresses sweet taste response in chorda tympani nerve recording in rodents and reduces mRNA expression of T1R3 in fungiform papillae (Okamoto et al., 2010), and restraint stress induced the translocation of glucocorticoid receptor into the nucleus in T1R3 expressing taste cells (Parker et al., 2014). Thus, it is suggested that decreased sweet receptor expression in taste cells via a negative glucocorticoid response element, if any, may contribute to dexamethasone-induced anorexia and/or anhedonia with decreased sweet sensation. However, in this study, daily dexamethasone did not
affect gene expression of sweet taste receptors T1R2 and T1R3 in the circumvallate papillae, revealing that dexamethasone-induced anorexia and anhedonia may not be accompanied with decreased sweet receptors expression in the taste cells. However, it is still not clear whether or not the dexamethasone-induced anorexia and/or anhedonia comprises a reduced sweet sensation, since we did not measure the physiologic response to sweet taste in this study.

**Fig. 5.** Q-PCR quantification of mRNA expression of sweet taste receptors T1R2 and T1R3 in the circumvallate papillae. Taste cells in the circumvallate papillae were harvested on the 4th and 28th day of dexamethasone treatment, respectively. Dexa 0.1; dexamethasone 0.1 mg/kg, Dexa 1.0; dexamethasone 1 mg/kg, n=6 in each group, Data are presented by means ± S.E.M.

Leptin has been suggested to modulate behavioral responses to sweet substances by influencing peripheral taste structures (Shigemura et al., 2004). Leptin is considered to be a stress hormone and its secretion is stimulated by stress (Hernandez et al., 2000; Konishi et al., 2006; Wallace et al., 2000). 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). Dexamethasone injection increases leptin mRNA expression in the adipose tissue (Lee et al., 2007), acutely increases the plasma level of leptin and induces long-lasting hyperleptinemia in rats (Caldefie-Chezet et al., 2001; Jahng et al., 2008). Leptin receptors in the brain reward circuit has been suggested to be implicated in symptoms of depression (Ates et al., 2014). Thus, it is expected that leptin may play a role not only in the central but also in the peripheral regulatory mechanisms underlying dexamethasone-induced anorexia and/or anhedonia. Further studies are warranted.

**In summary**

Daily dexamethasone injection suppressed food intake and weight gain in a dose-dependent manner, revealing its anorectic property. Dexamethasone-induced anorexia was followed by the development of anhedonia, referred by decreased sweet intake, and then behavioral depression during forced swim test. Daily dexamethasone did not affect gene expressions of T1R2 and T1R3 in the circumvallate papillae. It is concluded that the development of anhedonia, possibly caused by anorexia, is a pre-symptomatic feature of depression in rats treated with chronic dexamethasone, and its pathophysiologic mechanisms may not comprise changes in sweet receptors expression in taste cells.

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