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Role of oxidative stress in disrupting the function of negative glucocorticoid response element in daily amphetamine-treated rats

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ABSTRACT

Amphetamine (AMPH)-induced appetite suppression is associated with changes in hypothalamic reactive oxygen species (ROS), antioxidants, neuropeptides, and plasma glucocorticoid. This study explored whether ROS and glucocorticoid response element (GRE), which is the promoter site of corticotropin-releasing hormone (CRH) gene, participated in neuropeptides-mediated appetite control. Rats were treated daily with AMPH for four days, and changes in food intake, plasma glucocorticoid and expression levels of hypothalamic neuropeptide Y (NPY), proopiomelanocortin (POMC), superoxide dismutase (SOD), CRH, and glucocorticoid receptor (GR) were examined and compared. Results showed that food intake decreased and NPY gene down-regulated, while POMC, SOD, and CRH gene up-regulated during AMPH treatment. GR and GRE-DNA bindings were disrupted on Day 1 and Day 2 when glucocorticoid levels were still high. Pretreatment with GR inhibitor or ROS scavenger modulated mRNA levels in NPY, POMC, SOD and CRH in AMPH-treated rats. We suggest that disruptions of negative GRE (nGRE) on Day 1 and Day 2 are associated with an increase in oxidative stress during the regulation of NPY/POMC-mediated appetite control in AMPH-treated rats. These results advance the understanding of molecular mechanism in regulating AMPH-mediated appetite suppression.

1. Introduction

The hypothalamus is the major site to integrate appetite-controlled neurotransmitter and peripheral hormone in the control of energy metabolism. Several hunger and satiety mediators in the hypothalamus regulate appetite by controlling the activities of orexigenic neuropeptide Y (NPY)- and anorexigenic proopiomelanocortin (POMC)-containing neurons. Moreover, peripheral hormones, such as insulin, ghrelin and leptin, primarily bind and activate their cognate receptors directly in the hypothalamic arcuate nucleus or in the dorsal vagal complex in the medulla which communicates with the hypothalamus (Kim et al., 2014).

Amphetamine (AMPH) is a well-known appetite suppressant (Fleckenstein et al., 2007; Kuo et al., 2012; Chu et al., 2014); it acts by the central release of dopamine, which in turn down regulates NPY gene as well as up regulates POMC and melanocortin receptor 4 (MC4R) gene in the hypothalamus (Gillard et al., 1993; Chen et al., 2001; Hsieh et al., 2013, 2014). MC4R is a member of POMC system. AMPH can induce auto-oxidation of cytosolic dopamine and thus cause oxidative damage of dopamine terminals (Cadet et al., 2007), which is associated with increases in reactive oxygen species (ROS) (Kuo et al., 2011, 2015) and anti-oxidative enzyme, such as superoxide dismutase (SOD) and glutathione peroxidase in the hypothalamus (Frey et al., 2006; Tata and Yamamoto, 2007; Hsieh et al., 2015). Thus, AMPH can be regarded as a stressor (Schaefer et al., 2010; Grace et al., 2012). Moreover, AMPH can activate the hypothalamic–pituitary–adrenal (HPA) axis to increase plasma glucocorticoids (Schaefer et al., 2010), which may exert feedback inhibition on hypothalamus to decrease CRH release (Ostrander et al., 2006; Papadimitriou and Priftis, 2009).

The sequence of events leading to HPA activation by additive drugs appears to start within the brain, suggesting that central activation is not due to peripheral stimulation (Armario, 2010). Glucocorticoids released during stress have a wide impact on the brain through binding with glucocorticoid receptors (GR), a cytoplasmic protein (de Kloet et al., 2008). Glucocorticoid/GR complex can move to the nucleus and then interact with the glucocorticoid response element (GRE) located at the promoter site of the

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corticotrophin-releasing hormone (CRH) gene (Stahn et al., 2007; Lee et al., 2013). Previous evidence revealed that, in acute stress induced by additive drugs, an increase in CRH provides feedback inhibition by a mechanism of negative GRE (nGRE)-mediated control to inhibit CRH gene expression (Kyrou et al., 2006). However, in chronic stress and impaired GR conditions, both have been proposed to lead to the dysregulation of HPA axis activity (Simms et al., 2012).

In regard to the appetite control, acute stress exerts an anorexigenic effect through the stimulation of POMC neurons by increasing CRH gene expression and decreasing NPY secretion (Chrousos, 2000; Cadet et al., 2014). By contrast, chronic stress is associated with chronic activation of the HPA axis and prolonged glucocorticoid secretion, which exert orexigenic effects caused by the inhibition of CRH and the stimulation of NPY expression (Chrousos, 2000; Kyrou et al., 2006; Sobrino Crespo et al., 2014). It is unclear whether the chronically elevated levels of glucocorticoids during daily AMPH treatment can modulate nGRE-involved and NPY/POMC-mediated appetite control. We hypothesize that prolonged glucocorticoid secretion during daily AMPH treatment might disrupt nGRE-involved feedback inhibition due to an increase of oxidative stress in the hypothalamus in AMPH-treated rats.

2. Materials and methods

2.1. Animals treatments

Male Wistar rats weighing 200–300 g were obtained from the National Laboratory Animal Center (Taipei, Taiwan), which is certificated by Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International). The animals were individually housed in cages, were maintained at a temperature of 22 ± 2 °C in a room with a 12-h light-dark cycle (the light was turned on at 6:00 a.m. and turned off at 6:00 p.m.), and were habituated to frequent handling. Drugs were administered and food intake was determined daily at the beginning of the dark phase. Water and chow (LabDiet) were freely available to the rats throughout the experiment. Daily food intake amounted above 40 g/day were discarded because they indicated food spillage. All of the procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health. This study was approved by the Chung-Shan Medical University Experimental Animal Center (permit number 1489). All surgeries were performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

2.2. Experimental procedures

2.2.1. Protocol 1: effects of AMPH on food intake and hypothalamic gene expression

To examine the effect of AMPH (*d*-amphetamine) on feeding behavior, rats ($n = 8$ for each group) were injected intraperitoneally (i.p.) with the AMPH at a dose of 1, 2 or 4 mg/kg daily for four days. AMPH was first injected at the end of Day 0 (i.e., at the beginning of Day 1), and the amounts of daily food intake were calculated by accounting the difference of food amount between the previous day and the present day.

To determine the effect of daily AMPH (2 mg/kg, i.p.) on changes in hypothalamic NPY, POMC, MC4R, CRH, and SOD-1 gene expression, the rats were injected with the drug once a day for 1, 2, 3, or 4 days, depending on the rat group. On the sacrifice day, rats received a treatment of 2 mg/kg AMPH 40–50 min before being sacrificed to enhance the effects of the drug. Previous evidence reveals significant effects of AMPH on both central dopamine-associated locomotion (Kuczenski and Segal, 1989) and reductions in hypothalamic NPY expression (Kuo et al., 2001) are seen 40–50 min following treatment. The rats were anesthetized with 35–40 mg/kg pentobarbital and were then decapitated. Following decapitation, the hypothalamus was removed to determine the expression of protein or the levels of mRNA.

2.2.2. Protocol 2: effect of GR antagonist pretreatment on AMPH-induced responses

To investigate the effect of pretreatment with GR antagonist on AMPH-induced anorexia and hypothalamic NPY, POMC, CRH and SOD-1 mRNA levels, rats were treated daily with RU-486 (20 mg/kg; i.p.) 60 min before AMPH (4 mg/kg; i.p.) treatment. RU-486 (Mifepristone) is a highly selective GR antagonists (Simms et al., 2012), which can decrease AMPH-induced behavioral response via HPA stress axis (Aynara et al., 2010; Dustin et al., 2011) and may have a role in the treatment of a number of neuropsychiatric disorders (Peter and Allan, 2006). RU486 was suspended in 1% Tween-80 and 25% *b*-cyclodextrin in saline and stirred for 2 h before systemic injections (i.p.). For a desired concentration, RU486 was first dissolved in DMSO and then diluted with phosphate-buffered saline (PBS).

2.2.3. Protocol 3: effects of AMPH on GR expression and GR-GRE binding activity

To examine the effect of AMPH on GR expression and GR-GRE binding activity, rats were given AMPH (2 mg/kg; i.p.; $N = 6$ per group) daily for four consecutive days. At 40–50 min after daily AMPH treatment, the rats were sacrificed and the hypothalamus was removed to determine the GR expression and GRE/DNA binding activity by an electromobility shift assay (EMSA).

2.2.4. Protocol 4: effect of ROS scavenger pretreatment on AMPH-induced responses

To examine the effect of pretreatment with ROS scavenger on food intake and hypothalamic NPY, POMC, CRH, and SOD-1 expression in AMPH-treated rats, rats ($n = 6$–8 for each group) were infused daily with glutathione ethyl-ester (GSH-EE) (20 μl in concentration of 1 μmole/l; i.c.v.; the infusion rate was 4 μl/min) 40 min before AMPH treatment (4 mg/kg; i.p.) for 4 days. GSH-EE is a potent ROS scavenger that is particularly effective in restoring the mitochondrial glutathione redox state to a reduced state (GSH) (Benani et al., 2007). At 40–50 min after AMPH treatment, rats were anesthetized and the hypothalamus of each rat was removed from the brain and its mRNA levels were determined by qPCR. GSH-EE was dissolved in artificial corticospinal fluid (aCSF).

2.2.5. Protocol 5: to compare the difference of feeding in a 24-h AMPH treatment

To compare the differences in feeding behavior induced by a single treatment of AMPH (4 mg/kg; i.p.; N = 8 per group) between normal and AMPH-treated rats, the amounts of food intake were measured every 6 h over a 24-h period after drug treatment. There were four separate period of time to be recorded, i.e. 0–6 h, 6–12 h, 12–18 h, and 18–24 h, in the measure of 24-h feeding behavior.

2.2.6. Protocol 6: the effect of drug treatment on plasma corticosterone concentrations

To investigate the change of plasma corticosterone concentrations during daily AMPH (2 mg/kg, i.p.) injection, rats' blood samples were collected from Day 1 to Day 4 at 40–50 min after AMPH treatment. Tested groups included (1) AMPH-treated group, (2) GR inhibitor/AMPH co-administration group, and (3) ROS scavenger/AMPH co-administration group.