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Participation of ghrelin signalling in the reciprocal regulation of hypothalamic NPY/POMC-mediated appetite control in amphetamine-treated rats

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ABSTRACT

Hypothalamic neuropeptide Y (NPY) and proopiomelanocortin (POMC) have been documented to participate in amphetamine (AMPH)-induced appetite suppression. This study investigated whether ghrelin signalling is associated with changes in NPY/POMC-mediated appetite control. Rats were given AMPH daily for four days, and changes in food intake, body weight, plasma ghrelin, hypothalamic NPY, melanocortin 3 receptor (MC3R), ghrelin O-acyltransferase (GOAT), acyl ghrelin (AG) and ghrelin receptor (GHSR1a) were examined and compared. Food intake, body weight and NPY expression decreased, while MC3R expression increased and expressed reciprocally to NPY expression during AMPH treatment. Plasma ghrelin and hypothalamic AG/GOAT/GHSR1a expression decreased on Day 1 and Day 2, which was associated with the positive energy metabolism, and returned to normal levels on Day 3 and Day 4, which was associated with the negative energy metabolism; this expression pattern was similar to that of NPY. Infusion with a GHSR1a antagonist or an NPY antisense into the brain enhanced the decrease in NPY and AG/GOAT/GHSR1a expression and the increase in MC3R expression compared to the AMPH-treated group. Peripheral ghrelin and the central ghrelin system participated in the regulation in AMPH-induced appetite control. These results shed light on the involvement of ghrelin signalling in reciprocal regulation of NPY/POMC-mediated appetite control and may prove useful for the development of anti-obesity drugs.

1. Introduction

Ghrelin was first discovered in 1999 (Kojima et al., 1999), and has since been studied extensively for its role in the regulation of energy balance, feeding, stress, anxiety, and reward (Wellman & Abizaid, 2015a). Ghrelin is secreted in the stomach, and is the first peripheral hormone to be identified in the hypothalamic peptide system that exerts a dose-dependent orexigenic effect in obese and lean subjects (Druce et al., 2005; Kojima & Kangaw, 2010; Nakazato et al., 2001; Shintani et al., 2001). Moreover, ghrelin is also synthesized locally in the hypothalamus (Cowley et al., 2003), where it exerts a paracrine effect by activating the arcuate neuropeptide Y (NPY) neurons and inhibiting the proopiomelanocortin (POMC) neurons, leading to an increase in appetite (Chen et al., 2004). Ghrelin can be activated via acylation of the enzyme ghrelin O-acyltransferase (GOAT), which mediates the attachment of fatty acids to lipids and proteins (Gutierrez et al., 2008). The growth hormone secretagogue-receptor (GHS-R) is a ghrelin receptor that has two variants, GHS-R1a and GHS-R1b. GHS-R1a is predominantly expressed in several nuclei of the hypothalamus, such as arcuate nucleus (ARC) and dorsal medial hypothalamus (DMH) (Verhulst and Depoortere, 2012; Willesen, Kristensen, & Rømer, 1999). Three components of the ghrelin

Abbreviations: aCSF, artificial corticospinal fluid; AG, acyl ghrelin; ANOVA, analysis of variance; AMPH, amphetamine; ARC, arcuate nucleus; DMH, dorsal medial hypothalamus; ELISA, Blood collection and enzyme-linked immunosorbent assay; GOAT, ghrelin O-acyltransferase; GH, growth hormone GH; GHS-R, growth hormone secretagogue-receptor; ICV, intracerebroventricle; MC3R, melanocortin receptor 3; NPY, neuropeptide Y; IP, intraperitoneally; ODN, oligodeoxynucleotides; POMC, pro-opiomelanocortin; S-ODN, phosphorothioate oligodeoxynucleotides.

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system have been targeted in the research on energy metabolism: (1) acyl ghrelin (AG), which is an active acylated form of ghrelin; (2) GOAT, the enzyme that activates ghrelin and promotes it binding with its receptor; and (3) the ghrelin receptor-GHSR1a (Wellman & Abizaid, 2015a).

Amphetamine (AMPH) is a well-known appetite suppressant (Chu, Chen, Hsieh, Yu, & Kuo, 2014). The appetite-suppressing effect of AMPH is brought about via the central release of dopamine, which decreases NPY and increases POMC expression in the hypothalamus (Chen, Duh, Huang, Lin, & Kuo, 2001; Kuo et al., 2012). Orexigenic NPY and anorexigenic POMC present in the hypothalamus play a prominent role in the regulation of appetite (Rocha et al., 2014), and these two molecules may function reciprocally in the regulation of AMPH-induced appetite control (Hsieh, Chen, Yu, & Kuo, 2014). Melanocortin 3 receptor (MC3R) and MC4R are members of the POMC system, and activation of MC3R and MC4R are associated with an anorectic effect of AMPH (Cowley et al., 1999; Hsieh et al., 2014). In addition to the central neuropeptides, peripheral hormones also participate in the central control of appetite. Peripheral hormones, such as ghrelin, leptin and insulin, primarily bind with their receptors directly in the hypothalamus or in the dorsal vagal complex in the medulla, which communicates with the hypothalamus in the control of energy metabolism (Kim, Lin, Blomain, & Waldman, 2014).

Although ghrelin receptor is expressed more abundantly in the ARC (Perello et al., 2012) and participates in regulating food intake and energy balance (Currie, Mirza, Fuld, Park, & Vasselli, 2005; Kirchner, Heppner, & Tschop, 2012), it is unclear whether plasma ghrelin and hypothalamic AG/GOAT/GHS-R1a signalling are involved in the regulation of NPY/POMC-mediated appetite control in AMPH-treated rats. Moreover, as the result for the action of ghrelin antagonist on GHS-R1a is contrary; when peripheral administration, ghrelin antagonist may block growth hormone (GH) release in ARC, but may stimulate GH release in DMH (Halem et al., 2005; Hassouna et al., 2013). Thus, we will explore whether central administration of ghrelin antagonist can modulate ghrelin system and NPY/POMC expression, and the response of feeding behavior. In order to study this, we will examine the possible involvement of the ghrelin system-by central administration of either a GHS-R1a antagonist or an NPY antisense oligoneucleotide (i.e. NPY knockdown) in AMPH-treated rats.

2. Materials and methods

2.1. Animal treatments

Male Wistar rats weighing 200–300 g were obtained from the National Laboratory Animal Center (Taipei, Taiwan). 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, PMI Nutrition, International, Brentwood, MO, USA) were freely available to the rats throughout the experiment. Data points 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 (permission number 1489). All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

2.2. Experimental procedures

To examine the effect of AMPH (*d*-amphetamine) on feeding behavior and body weight, 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 intake data were calculated with respect to the food amount of the previous day.

To determine the effect of daily AMPH (2 mg/kg, i.p.) on changes in hypothalamic NPY, MC3R, AG, GOAT and GHS-R1a expression, the rats ($N = 8$ per group) 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 50 min before being sacrificed to enhance the effects of the drug. The rats were anesthetized with 35–40 mg/kg pentobarbital and were then decapitated. Following decapitation, the hypothalamus was removed to detect the expression of protein using the technique of Western Blot (for NPY, MC3R, GOAT and GHS-R1a analysis) or ELISA (foe AG analysis).

To determine the effect of GHSR1a antagonist (BIM-28163) on AMPH-induced anorexia, body weight change and on the changes of hypothalamic NPY, MC3R, AG, and GOAT levels, rats ($N = 6–8$ for each group) were i.c.v. pretreated with BIM-28163 (5 μg in a 10-μl vehicle; i.c.v.) at 30 min before 2 mg/kg AMPH treatment. Rats received BIM-28163 and/or AMPH at 40–50 min prior to being anesthetized with 35–40 mg/kg pentobarbital and decapitated to remove hypothalamus. BIM-28163 is a selective GHSR1a antagonist, which does not interact with a wide variety of known receptors involved in weight regulation (Xu et al., 2013). Moreover, although BIM-28163 has sequence with approximately 86% homology to the natural ghrelin, it can prevent growth hormone release (Halem et al., 2005). BIM-28163 alone had no significant effect on daily food intake. BIM-28163 was dissolved in the solution of artificial CSF.

To assess the effect of pretreatment with NPY antisense oligodeoxynucleotide (ODN) on the anorectic response and body weight change of AMPH, rats ($N = 8$ per group) were given intracerebroventricularly (i.c.v.) NPY antisense (20 μg in a 10-μl vehicle) 1 h before AMPH (2 mg/kg; i.p.) treatment. Before drugs treatment, rats were i.c.v. administered a similar dose of NPY antisense daily for 2–3 days until the feeding behavior was slightly reduced. The response is due to the fact that either continuous or repeated i.c.v. injections of antisense may be necessary to maximize behavioral effects and importantly to block the synthesis of a constitutively active gene product (Ogawa & Pfaff, 1998; Zhang & Creese, 1993).

To examine the effect of NPY antisense (or missense) on hypothalamic NPY, MC3R, AG, GOAT and GHS-R1a expression, rats ($N = 8$) were i.c.v. infused with antisense or missense (20 μg in a 10-μl vehicle; i.c.v.) 1 h before AMPH treatment. At 50 min after NPY antisense and/or AMPH treatment, rats were anesthetized and the hypothalamus of each rat was removed from the brain and its desired peptide or protein were determined.

2.3. Blood and tissue collection for enzyme-linked immunosorbent assay (ELISA)

To investigate the concentrations of plasma and hypothalamic AG during daily AMPH injection, rats were sacrificed and blood samples were collected under light anesthesia with ether from Day 1 to Day 4 at 50–60 min after AMPH treatment. Blood and hypothalamic samples were collected in tubes containing EDTA and protease inhibitors to prevent the degradation of AG. Blood were centrifuged at 3000 rpm for 15 min. Plasma was removed and frozen at −20 °C for subsequent biochemical determinations. Hypothalamus were quickly collected and frozen by liquid nitrogen.