科技部補助專題研究計畫成果報告
期末報告

厭食劑在腦內之分子機制：NPY 受器、GRE-DNA 結合位及 STAT3 轉錄因子之角色(第 3 年)

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中 華 民 國 104 年 09 月 17 日

中 文 摘 要 ： 除了 POMC (proopiomelanocortin)，我們也發現：安非他命之厭食作用透過下室丘 NPY(neuropeptide Y) 、NPY receptor 1 (Y1R)及 CART(cocaine and amphetamine related transcript)神經傳遞系統調控,且 CART 與 NPY、Y1R 有反向交互控制的現象,本實驗進一步證明 NPY knockdown 與 Y1R inhibition 二種處理方式，可調控下室丘 CART 媒介食欲之調控

中文關鍵詞： NPY, CART, Y1R, POMC

英 文 摘 要 ： Amphetamine (AMPH)-induced appetite suppression has been attributed to its inhibition of neuropeptide Y (NPY)-containing neurons in the hypothalamus. This study examined whether hypothalamic cocaine- and amphetamine-regulated transcript (CART)-containing neurons and NPY Y1 receptor (Y1R) were involved in the action of AMPH. Rats were treated daily with AMPH for four days, and changes in feeding behavior and expression levels of NPY, CART, and POMC were assessed and compared. The results showed that both feeding behavior and NPY expression decreased during AMPH treatment, with the biggest reduction occurring on day 2. By contrast, the expression of CART and melanocortin 3 receptor (MC3R), a member of the POMC neurotranamission, increased with the maximum response on day 2, directly opposite to the NPY expression results. The intracerebroventricular infusion of NPY antisense or Y1R inhibitor both modulated AMPH-induced anorexia and the expression levels of MC3R and CART. The results suggest that in the hypothalamus both POMC- and CART-containing neurons participate in regulating NPY-mediated appetite control during AMPH treatment. These results may advance the knowledge of molecular mechanism of anorectic drugs.

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Both neuropeptide Y knockdown and Y1 receptor inhibition modulate CART-mediated appetite control

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ABSTRACT

Amphetamine (AMPH)-induced appetite suppression has been attributed to its inhibition of neuropeptide Y (NPY)-containing neurons in the hypothalamus. This study examined whether hypothalamic cocaine- and amphetamine-regulated transcript (CART)-containing neurons and NPY Y1 receptor (Y1R) were involved in the action of AMPH. Rats were treated daily with AMPH for four days, and changes in feeding behavior and expression levels of NPY, CART, and POMC were assessed and compared. The results showed that both feeding behavior and NPY expression decreased during AMPH treatment, with the biggest reduction occurring on Day 2. By contrast, the expression of CART and melanocortin 3 receptor (MC3R), a member of the POMC neurotransmission, increased with the maximum response on Day 2, directly opposite to the NPY expression results. The intracerebroventricular infusion of NPY antisense or Y1R inhibitor both modulated AMPH-induced anorexia and the expression levels of MC3R and CART. The results suggest that in the hypothalamus both POMC- and CART-containing neurons participate in regulating NPY-mediated appetite control during AMPH treatment. These results may advance the knowledge of molecular mechanism of anorectic drugs.

Introduction

The hypothalamus is the major site of integration of anorexigenic and orexigenic signaling. Within the arcuate nucleus there exist two main populations of neurons: those expressing the orexigenic neuropeptide Y (NPY) or agouti-related peptide (AgRP)-expressing neurons, and those expressing the anorexigenic proopiomelanocortin (POMC) or cocaine- and amphetamine-regulated transcript (CART)-expressing neurons. Several satiety hormones induce their anorexic effects by either inhibiting the activity of NPY/AgRP neurons or activating POMC/CART neurons. In addition, peripheral satiety hormones, such as ghrelin and leptin, primarily bind and activate their cognate receptors directly in the arcuate nucleus, or in the dorsal vagal complex in the medulla, which communicates with the hypothalamus (Gilbert et al., 2014).

Feeding behavior is modulated mainly in the brain by several neural and hormonal inputs into the hypothalamus (Parker and Bloom, 2012; Yada et al., 2012), and the NPY neural pathway has a prominent role in appetite regulation (Rocha et al., 2014). NPY is a highly conserved neuropeptide that regulates several physiological responses, such as feeding behavior, energy balance (Sahu et al., 1997; Mercer et al., 2011; Younes-Rapozo et al., 2012), emotional processing (Stadlbauer et al., 2013), and prevention of oxidative stress in the brain (dos Santos et al., 2013). Electro-physiologically, NPY robustly inhibits hypothalamic neurons in the ventromedial nucleus, which can produce an anorexigenic output signal by hyperpolarizing the neurons and decreasing their ability to fire action potentials (Chee et al., 2010).

The mechanism underlying the appetite-suppressing effect of amphetamine (AMPH) is associated with the central release of dopamine, which decreases the expression of NPY and increases the expression of POMC in the hypothalamus (Chen et al., 2001; Kuo, 2006; Hsieh et al., 2013b). It has been reported that NPY and POMC peptides might function reciprocally in the regulation of appetite suppression in rats treated with phenylpropanolamine, an AMPH-like anorectic drug (Hsieh et al., 2013b). Although NPY neurons can suppress POMC transmission during the control of energy balance, it is still unknown whether the CART neural system, which is another anorexigenic drive, is involved in regulating NPY-mediated appetite suppression in AMPH-treated rats. We hypothesized that cerebral dopamine, POMC- and CART-containing neurons might be involved in the regulation of NPY-mediated appetite control in AMPH-treated rats.

The CART neuropeptide was initially identified as a result of its positive regulation by the psychomotor stimulants cocaine and amphetamine (Kuhar and Dall Vechia, 1999). Numerous studies have established the role of CART in food intake, maintenance of body weight,

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endocrine control (Hunter et al., 2004; Rogge et al., 2008), drug-related reward and addiction (Vicentic and Jones, 2007), and anxiety control (Wiehager et al., 2009). It was demonstrated recently that CART exhibits cerebroprotective effects against ischemic stroke and β-amyloid protein neurotoxicity (Zhang et al., 2012). Anatomically, the colocalization of CART with both orexigenic and anorexigenic neuropeptides suggests that CART-containing neuron has a modulatory role in feeding behavior (Koylu et al., 1998; Broberger, 1999; Sarkar et al., 2004). In addition, there are functional interactions between CART- and NPY-containing neurons in the hypothalamus, as neural varicosities containing NPY may form dense pericellular baskets around CART-immunoreactive cell bodies (Lambert et al., 1998). Moreover, the CART peptide is a modulator of dopamine and psychostimulants, because CART tends to oppose large increases in dopamine signaling (Hubert et al., 2008). Thus, it is possible that CART may participate in regulating NPY- and POMC-mediated appetite control in AMPH-treated rats. There are two active CART peptide fragments, CART (55–102) and CART (62–102), that exhibit different relative activities in different testing paradigms (Kimmel et al., 2002). CART (55–102) is five-fold more potent than CART (62–102) in the inhibition of food intake (Vicentic et al., 2006).

The melanocortins, a family of peptides produced from the POMC gene, regulate ingestive behavior and energy expenditure (Butler, 2006). Melanocortins mediate their effects through a family of five related G protein-coupled melanocortin receptors (MCR), i.e., MC1R through MC5R. In the CNS, melanocortin are agonists of the MC3R and MC4R (Cone, 2005). Thus, obesity can result from deletion of either MC4R (Huszar et al., 1997) or MC3R (Butler et al., 2000) in rodent animals. MC3R has been demonstrated to produce potent effects on food intake, body weight and energy expenditure (Fan et al., 2000). Thus, we hypothesized that hypothalamic CART (55–102) might be involved in the reciprocal regulation between NPY and MC3R, during the control of AMPH-induced anorexia.

NPY acts on at least five receptors, which include the Y1, Y2, Y4, Y5, and y6 subtypes (Michel et al., 1998). Of these NPY receptors, Y1 receptor (Y1R) and Y5 receptor (Y5R) have been suggested to mediate the effect of NPY on feeding (Gerald et al., 1996; Antal-Zimanyi et al., 2008). Previous reports showed that inhibition of NPY Y1 receptor (Y1R), but not Y5R, can modify AMPH-induced anorexia and the expression levels of hypothalamic NPY and POMC during AMPH treatment (Kuo et al., 2012a; Hsieh et al., 2013a,b). However, it is still unclear if this Y1R inhibition can also modulate CART expression during AMPH treatment. We hypothesized that neural signaling via Y1R might mediate central effects of AMPH in the regulation of hypothalamic NPY, POMC and CART expression.

Taken together, the main aims of this study were to investigate, in AMPH-treated rats, (1) whether the CART neurotransmission is involved in the reciprocal regulation between POMC- and NPY-mediated appetite control; (2) whether NPY-Y1R neurotransmission participates in CART-mediated appetite control; and (3) whether central dopamine mediated CART neurotransmission.

Materials and methods

Animals

Male Wistar rats weighing 200–300 g were obtained from the National Laboratory Animal Center in Taiwan, ROC. The animals were housed individually in cages and maintained at a temperature of 22 ± 2 °C in a room with a 12-h light–dark cycle (lights on at 6:00 a.m.). The rats were also habituated to frequent handling. Drugs were administered and food intake was determined every day at the beginning of the dark phase (6:00 p.m.). Water and chow (LabDiet) were freely available throughout the experiment. Food intake data points above 35 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 and reviewed by the National Science Council, Taiwan, R.O.C.

Drugs, chemicals, and reagents

Chow (LabDiet) was purchased from PMI Nutrition International (Brentwood, MO, USA). AMPH, angiotensin II, haloperidol, and Tris–HCl were purchased from Sigma-Aldrich (St. Louis, MO, USA). Antibodies against NPY and β-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). MC3R antibody was from Gibco BRL, Life Technologies, Inc. (Rockville, MD, USA), while CART (55–102) and CART (62–102) antibodies were from Phoenix Pharmaceuticals, Inc. (Burlingame, CA, USA). Quantitative real-time PCR analysis was performed using a Taqman One-step PCR Master Mix (Applied Biosystems, USA). TRIZOL reagent (Life Technologies, Inc., Grand Island, USA) was used in tissue homogenization. Antisense and DNA primer were synthesized by Proligo Pty Ltd. (Singapore).

Animal treatment

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 AMPH at a dose of 2 or 4 mg/kg daily for 4 days. AMPH was first injected at the end of Day 0 (i.e. at 6:00 p.m. or at the beginning of Day 1). The food intake and body weight data were calculated with respect to the food amount and body weight of the previous day. Rats received AMPH 40 min before being anesthetized (pentobarbital, 35 mg/kg; i.p.) and decapitated. To examine the effect of dopamine receptor antagonist (DRA) pretreatment on AMPH-induced feeding behavior and body weight change, rats were i.p. injected with 1 mg/kg haloperidol (Hal) 40 min before 2 mg/kg AMPH treatment once a day for 4 days. Our previous reports revealed that AMPH treatment increased the brain dopamine concentration (Kuo and Cheng, 2002) and that AMPH-induced anorexia showed a significant change only at the initial 0–6 h time interval in a 24-h test period (Kuo, 2005). Hal is a non-selective dopamine D1/D2 receptor antagonist, which can block AMPH-induced anorexia during a 24-h test period (Chen et al., 2001).

To determine the effect of daily AMPH (2 mg/kg; i.p.) on the changes in hypothalamic NPY, CART, and POMC mRNA levels, rats were injected with the drug once a day for 1, 2, 3, or 4 days, depending on the group. Similarly, to determine the effect of daily AMPH (2 mg/kg; i.p.) on the changes in hypothalamic NPY, CART (55–102), CART (62–102), and MC3R expression, rats were injected with the drug once a day for 1, 2, 3, or 4 days, depending on the group. On the sacrifice day, rats received a treatment of 2 mg/kg AMPH 40 min before being sacrificed to enhance the effects of the drug. The rats were anesthetized with 30 mg/kg pentobarbital and decapitated. Following decapitation, the hypothalamus was removed to determine mRNA or protein expression. The rat's hypothalamus was removed from the brain immediately and subjected to determinations of mRNA levels or protein expression, or stored at −80 °C until its use for analysis.

To determine the effect of DRA pretreatment on the changes of NPY, CART, and POMC mRNA levels in the hypothalamus in AMPH-treated rats on Day 1, rats ($N = 8$ each group) were injected with 1 mg/kg Hal 40 min before 2 mg/kg AMPH treatment. Rats received Hal and/or AMPH at 40 min prior to being i.p. anesthetized with 30 mg/kg pentobarbital and decapitated to remove hypothalamus from the brain immediately, which was then subjected to determinations of protein levels or stored at −80 °C until further use.

To assess the effect of pretreatment with NPY antisense oligodeoxynucleotide (ODN) on the anorectic response 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 (4 mg/kg; i.p.) treatment daily for 4 days. Before AMPH treatment, rats were i.c.v. administered a similar dose of antisense daily for 2–3 days until the

feeding behavior response 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 (Zhang and Creese, 1993; Ogawa and Pfaff, 1998). The description of the surgery for i.c.v. cannulation and the information of antisense were described in the desired section.

To examine the effect of NPY antisense (or missense) on NPY, MC3R, and CART (52–102) expression levels in AMPH-treated rats, rats (N = 6–8 for each group) were infused daily with antisense or missense (20 μg in a 10-μl vehicle; i.c.v.) at 1 h before daily treatment with 2 mg/kg AMPH for 4 days. Before AMPH treatment, rats were i.c.v. infused with a similar dose of antisense (or missense) daily for 2–3 days until the response of feeding behavior was reduced slightly in the antisense group. At 40 min after antisense (missense) and/or AMPH treatment, rats were anesthetized and the hypothalamus of each rat was removed from the brain and its NPY, MC3R, and CART (52–102) expression levels were determined by Western Blot.

To determine the effect of Y1R antagonist on AMPH-induced anorexia and on the changes of hypothalamic NPY, MC3R, and CART (55–102) levels, rats (n = 6–8 for each group) were pretreated with BIBP-3226 at 30 min before the 4 mg/kg AMPH treatment. BIBP-3226 is developed as an Y1R antagonist, which is known not to have any effect at the Y2, Y4, and Y5 receptors (Rudolf et al., 1994) and can significantly reduce NPY-induced feeding (O'Shea et al., 1997). We therefore studied the effect of BIBP-3226 (80 nmol, i.c.v.; MW 473.6) on a 24-h AMPH-induced feeding response and on the changes of NPY, MC3R, and CART (55–102) levels. Rats received BIBP and/or AMPH at 40 min prior to being i.p. anesthetized with 30 mg/kg pentobarbital and decapitated to remove hypothalamus. BIBP-3226 had no significant effect on daily food intake (decrease about 10% compared to saline-treated group). It was for this reason that we allowed a 30-min period between injection of BIBP-3226 and the stimulant of feeding. The first injection of AMPH was conducted at the end of the light period (i.e., at 6:00 p.m.). The intake data were calculated as the total amount of food during the previous day.

RNA extraction

Hypothalamic NPY, POMC, and CART mRNA levels were measured in a block of mediobasal hypothalamic tissue as described previously (Magni and Barnea, 1992). In brief, total RNA was isolated from this block using the modified guanidinium thiocyanate–phenol–chloroform method (Chomczynski and Sacchi, 1987). Each hypothalamic block was homogenized in 1 ml of TRIZOL reagent (Life Technologies, Inc., Grand Island, USA) using an Ultrasonic Processor (Vibra Cell, Model CV17; Sonics & Materials Inc., Danbury, Connecticut, USA). After incubation at 22 °C for 5 min, 0.2 ml of chloroform was added to each sample, shaken vigorously for 15 s, incubated at 22 °C for 3 min, then centrifuged at 12,000 × g for 15 min at 4 °C. After removal of aqueous phase and precipitation with 0.5 ml isopropanol, samples were incubated at 22 °C for 10 min and centrifuged at 12,000 × g for 15 min at 4 °C. The gel-like RNA pellets were washed with 75% ethanol by vortexing and centrifugation at 7500 × g for 5 min at 4 °C. Thereafter, RNA pellets were dried briefly, dissolved in RNase-free water, and stored at −80 °C. The content of RNA was determined spectrophotometrically at 260 nm (Hitachi U-3210, Japan).

Quantitative real-time polymerase chain reaction (qPCR)

The total RNA was isolated from the hypothalamus using TRIZOL (Life Technologies, Grand Island, NY, USA) as described above. The qPCR analysis was performed using a Taqman One-step PCR Master Mix (Applied Biosystems, CA, USA). Briefly, 100 ng of cDNA was added per 25 μl reactions with each NPY, POMC and CART, or GAPDH primer and TaqMan probes. The qPCR assays were performed in triplicate on a StepOnePlus sequence detection system. The oligonucleotide sequences of TaqMan probes and primers were used according to the manufacturer's instructions. The threshold was set above the non-template control background and within the linear phase of the target gene amplification to determine the cycle number at which the transcript was detected.

Lateral ventricular cannulation

Stereotaxic surgery (Kopf Model 900, Tujunga, CA, USA) was performed on each rat under pentobarbital anesthesia (30 mg/kg, i.p.). The target of cannulation was near the junction of the right lateral ventricle and the third ventricle (coordinates: 0.8 mm posterior to the Bregma, 1.5 mm from the midline, and 3.5–4.0 mm below the dura) (Paxinos and Watson, 1986). A 23-g stainless steel guide cannula was implanted and secured to the skull using stainless steel screws and dental cement. The correct placement was confirmed by observing the transient and rapid inflow of the vehicle in polyethylene tubing connected to a 28-g injector cannula. The cannula was then occluded with a 28-g stylet. For the infusion of antisense, the stylet was replaced with a 28-g injector cannula extending 0.5 mm below the tip of the guide cannula. For all experiments, the cannula placement was verified by histochemistry of brain section and by the administration of angiotensin II (100 ng/rat). Angiotensin II reliably induced water drinking in non-deprived rats when administered into the cerebroventricles (Ritter et al., 1981). Only data from rats that drank more than 10 ml of water in 30 min were included in this study. Behavioral testing of drinking began about 1 week after the cannulation surgery and the restoration of feeding behavior, and then angiotensin II was administered to confirm the cannula placement. It was about two days after the treatment of angiotensin II to confirm the restoration of normal drinking behavior, and then we started the experiment of AMPH treatment (Day 0).

Cerebral infusion of NPY antisense

An 18-mer oligonucleotide (ODN) near the initiation codon encompassing bases 10–27 of the rat NPY mRNA sequence (GenBank accession no. 15880) was selected. The antisense ODN (5′-CCCCATTC GTTTGTTACC) is inversely complementary to this sequence. Phosphorothioate internucleotide linkages were obtained through treatment with tetraethylthiuram disulfide, and the resulting phosphorothioate oligonucleotides were purified and lyophilized. An 18-mer missense ODN (5′-TTATTCCCCCAGTTTGCC) was used as the control. As described previously (Gillard et al., 1993), this antisense sequence did not appear to display self-hybridization; therefore it was effective in blocking the message read-through. In addition, one week of daily i.c.v. injection of this antisense appeared to reduce food intake and body weight as compared with the missense-treated control. Rats were handled and i.c.v. injected with vehicle 4 days prior to the experimental injections to accustom them to the procedure. One hour before AMPH (2 mg/kg/day, i.p.) treatment, antisense ODN (10 μg/10 μl/day) was administered to 8 rats, and the same treatment was repeated for 7 days. An equivalent dose of missense ODN was administered to each of the 8 rats that served as the control. Food intake and body weight changes were recorded daily. We used NPY antisense that was phosphorothioate-modified (S-ODNs) only on the three terminal bases of both the 5′ and 3′ ends, because these S-ODNs can improve hybridization affinity and nuclease resistance and were regarded as a well-established agent in several vertebrate systems (Ogawa and Pfaff, 1998) and rat brain (Kuo et al., 2012a, 2012b). Both antisense and missense S-ODNs were dissolved in artificial corticospinal fluid (aCSF) containing 140 mM NaCl, 3.35 mM KCl, 1.15 mM $MgCl_2$, 1.26 mM $CaCl_2$, 1.2 mM Na_2HPO_4 and 0.3 mM NaH_2PO_4; pH 7.4.

Western Blotting

Hypothalamus tissues were lysed using a cold mammalian protein extraction buffer kit (GE Healthcare Bio-Sciences Corp., Piscataway, NJ) with protease inhibitor cocktails for 20 min to prepare the total cell lysates. The suspension was vortexed and centrifuged at 14,000 $\times g$ for 1 min at 4 °C. Samples were subjected to electrophoresis. Proteins were separated on a 12.5% SDS polyacrylamide gel (for NPY, MC3R, MC4R, and β-actin) or 15% SDS polyacrylamide gel (for CART), which were transferred onto a nitrocellulose membrane, and incubated with specific antibodies against NPY (1:1000 dilution), CART (1:500 dilution), MC3R (1:1000 dilution), and β-actin (1:1000 dilution). After incubation with horseradish peroxidase goat anti-rabbit IgG, the color signal was developed using 4-chloro-1-naphthol/3,3′-diaminobenzidine and 0.9% (w/v) NaCl in Tris–HCl. The relative photographic density was quantified by scanning the photographic negative film on a Gel Documentation and Analysis System (AlphaImager 2000, Alpha Innotech Corporation, San Leandro, CA, USA).

Statistical analysis

A two-way or one-way analysis of variance (ANOVA) followed by Dunnett's test ($p < 0.05$) was used to detect significant differences between the groups. ANOVA followed by the least-significant difference (LSD) post hoc test, was used to analyze the results, and the paired-samples *t* test were used to analyze within subject differences. ANOVA with LSD post hoc test was applied for analyzing independent data. Effect sizes were calculated using Eta squared (η^2) following the main and interaction effects, and Cohen's *d* reported for all pairwise comparisons. Data are presented as the mean ± SEM. Statistical significance was set at $p < 0.05$.

Results

The effect of AMPH and/or DRA on feeding behavior and body weight

Changes of feeding behavior in rats receiving AMPH and/or DRA treatment were shown in the upper panel of Fig. 1. The repeated measures of general linear model showed significant differences of the treatments with AMPH and/or DRA, compared with the control. There were time effects [$F(4,140) = 48.37$, $p < 0.001$], treatment effects [$F(4,35) = 10.09$, $p < 0.001$], and time-by-treatment interactions [$F(16,140) = 11.70$, $p < 0.001$]. Results for the effect size analysis revealed that all partial $\eta^2 > 0.54$. Post hoc test by LSD showed lowered food intake in the groups receiving AMPH at both of dosages of 2 and 4 mg/kg [both $p < 0.05$], compared with the control group. But the groups treated with DRA and DRA+AMPH showed no difference in food intake, compared with the controls. Furthermore, paired comparisons revealed that reduction of food intake was observed on Days 1–3 after 2 mg/kg of, and on Days 1–4 after 4 mg/kg of, AMPH treatment [all Cohen's $d > 1.02$], compared with Day 0. Moreover, the effect of 2 mg/kg AMPH on Day 4 had a significant effect compared to that on Day 2 ($p < 0.05$), revealing that 2 mg/kg AMPH could induce gradually the tolerant effect to AMPH. Therefore, AMPH at a dose of 2 mg/kg was employed for the subsequent measures since after two days there was a restoration of food intake.

Changes in body weight in rats receiving AMPH and/or DRA treatment are shown in Fig. 6. Results revealed that the decreases of daily body weight in AMPH and/or DRA-treated rats were expressed in a pattern similar to the decreases of daily food intake. Daily pretreatment with DRA could result in restoration of food intake and body weight change, revealing the involvement of dopamine D1/D2 receptors in the regulation of AMPH-induced anorexia during a 4-day testing period.

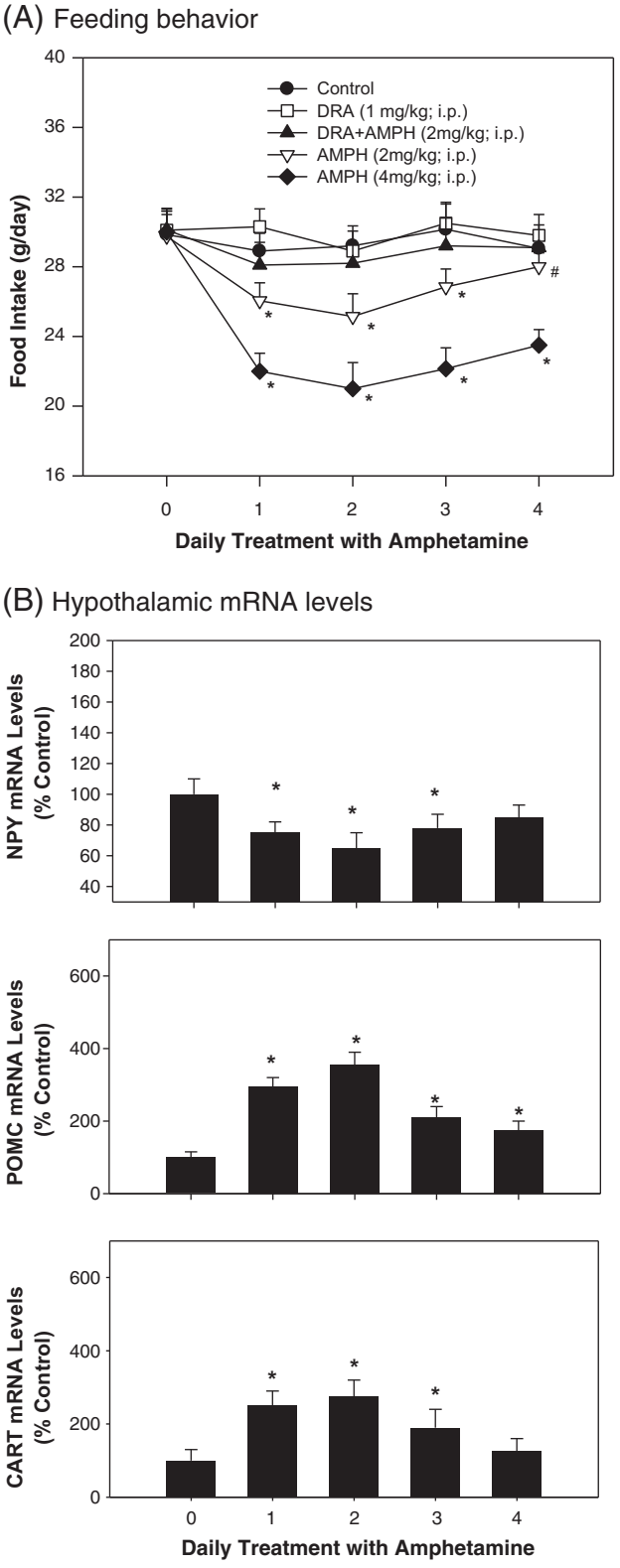

Fig. 1. (A) The effect of DRA and/or AMPH treatment on daily food intake over a 4-day period. DRA (1 mg/kg; i.p.) was administered to rats 40 min before 2 mg/kg/day AMPH once a day (at 6:00 p.m. of each day) for 4 days. The first injection of AMPH (2 and 4 mg/kg; i.p.) was conducted at the end of Day 0. Each point represents the mean ± SEM of 8 rats. $^{*}p < 0.05$ vs. the control group. $^{\#}p < 0.05$ vs. the AMPH (2 mg/kg)-treated group on Day 2. (B) Effect of AMPH (2 mg/kg; i.p.) on hypothalamic NPY, POMC, and CART mRNA levels. Results showed the relative densitometric value for the analysis of quantitative real-time PCR products. Bars were mean ± SEM. $N = 6$ each group. $^{*}p < 0.05$ vs. control.

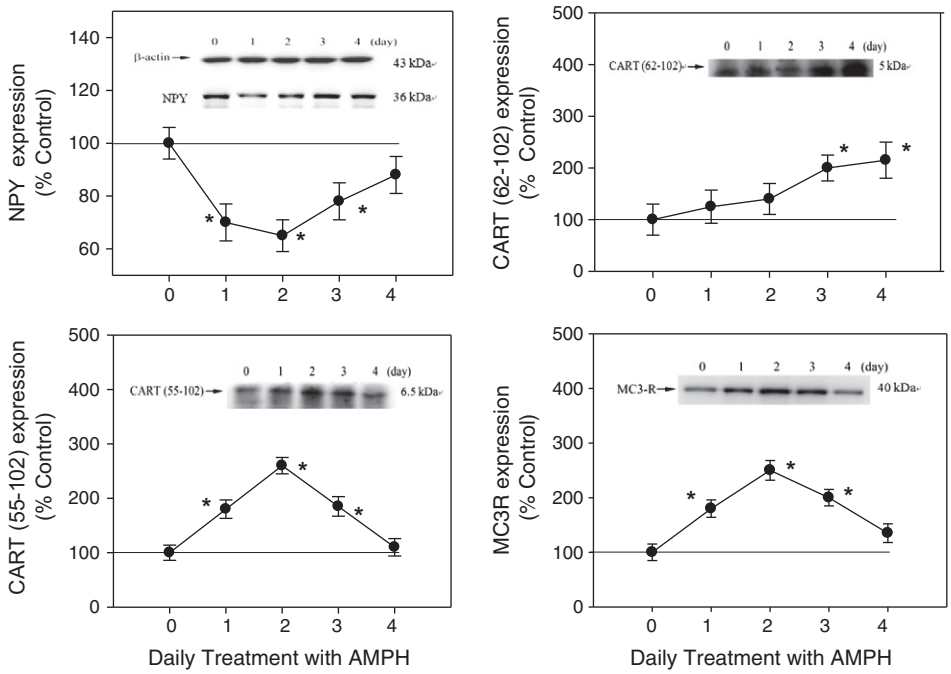

Fig. 2. Effects of daily AMPH on the expression of hypothalamic NPY, CART (55–102), CART (62–102), MC3R, and β-actin over a 4-day period. Upper panel: the results of Western Blot. Lower panel: relative densitometric values for Western Blot in control and 2 mg/kg AMPH-treated groups. Contents of protein in AMPH-treated groups were indicated as the percentage of the control group. Bars are the mean ± SEM. $N = 8$ each group. $^{*}p < 0.05$ vs. control. $^{\#}p < 0.05$ vs. the AMPH-treated groups.

The effect of AMPH and/or DRA on NPY, POMC, and CART mRNA levels

Results shown in the lower panel of Fig. 1 revealed that daily AMPH increased POMC, and CART mRNA levels with the maximum response on Day 2, which was opposite to the decrease of NPY mRNA level with the biggest reduction on Day 2. Using GAPDH as the internal standard, the ratio of NPY, POMC, and CART mRNA over GAPDH in each group was calculated and compared. Analysis with one-way ANOVA revealed a significant difference in NPY mRNA level [$F(4,29) = 4.76$, $p = 0.005$], and LSD post hoc test showed lower NPY mRNA levels on Days 1–3 [all $p < 0.05$, all Cohen's $d > 1.47$], compared to control. Differences in POMC mRNA levels were observed [$F(4,29) = 31.52$, $p < 0.001$], and LSD post hoc test showed higher POMC mRNA levels on Days 1–4 [all $p < 0.01$, all Cohen's $d > 2.60$], compared to the control. In addition, differences in CART mRNA levels were observed [$F(4,29) = 32.50$, $p < 0.001$], and LSD post hoc test showed higher CART mRNA levels on Days 1–3 [all $p < 0.001$, all Cohen's $d > 3.87$], compared to the control.

Results shown in Fig. 7 revealed that pretreatment with DRA in AMPH-treated rats could result in restoration of NPY, CART and POMC mRNA levels on the first day (Day 1) of drug treatment. Statistical analysis with one-way ANOVA indicated a decrease of NPY mRNA level [$F(3,23) = 4.91$, $p < 0.01$, $\eta^2 = 0.42$] but increases in CART mRNA level [$F(3,23) = 20.42$, $p < 0.001$, $\eta^2 = 0.75$] and POMC level [$F(3,23) = 35.74$, $p < 0.001$, $\eta^2 = 0.84$] in AMPH-treated group compared to the control (Day 0). Moreover, results also indicated the effect of DRA pretreatment on the restoration of NPY, CART and POMC mRNA levels back to normal level compared to the AMPH-treated group. This result revealed that DRA treatment could modulate the effects of AMPH on NPY, CART and POMC mRNA levels, which might be associated with the anorectic effect of AMPH.

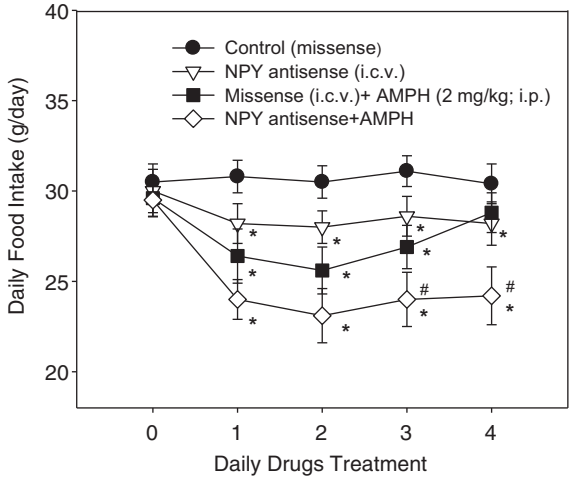

Fig. 3. The effect of NPY antisense (or missense) pretreatment on daily AMPH-mediated food intake over a 4-day period. Daily missense or antisense treatment (20 μg/10 μl/day, i.c.v.) was administered 1 h before daily AMPH treatment. $^{*}p < 0.05$ vs. the missense groups. $^{\#}p < 0.05$ vs. the AMPH-treated groups of each treatment. Bars are the mean ± SEM. $N = 8$ per group.

The effect of AMPH on NPY, MC3R, and CART expression

Results shown in Fig. 2 revealed that daily AMPH treatment decreases NPY but increased MC3R, CART (55–102) and CART (62–102) expression during AMPH treatment. Using β-actin as the internal standard, the ratio of NPY, MC3R, CART (55–102), and CART (62–102) over β-actin in each group was calculated and compared. Analysis with one-way ANOVA revealed a decrease in NPY from Day 1 to Day 3 [$F(4,25) = 2.85$, $p < 0.05$, $\eta^2 = 0.37$], but revealed increases in MC3R expression from Day 1 to Day 3 [$F(4,25) = 3.11$, $p < 0.05$, $\eta^2 = 0.47$], CART (55–102) expression from Day 1 to Day 3 [$F(4,25) = 3.56$, $p < 0.05$, $\eta^2 = 0.51$], and CART (62–102) expression from Day 3 to Day 4 [$F(4,25) = 2.23$, $p < 0.05$, $\eta^2 = 0.29$] compared to the control. These results revealed that MC3R and CART (55–102) expression were increased with maximum response on Day 2 during AMPH treatment, which was opposite to the expression of NPY expression with the biggest reduction on Day 2. However, the expression of CART (62–102) was in a distinct pattern compared to other groups.

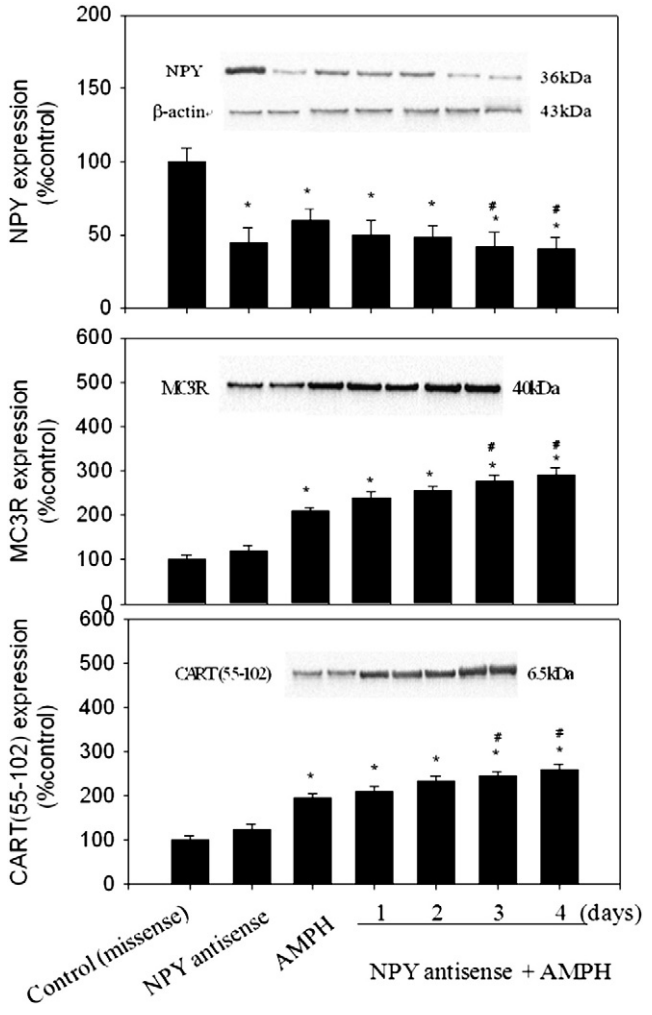

Fig. 4. The effect of NPY antisense (or missense) pretreatment on AMPH-induced changes of NPY, CART (55–102), MC3R, and β-actin expression over a 4-day period. Daily missense or antisense treatments (20 μg/10 μl/day, i.c.v.) were administered 1 h before daily 4 mg/kg AMPH treatment. Results showed that NPY antisense could partially reverse CART (55–102) and MC3R expression back to normal. $^{*}p < 0.05$ vs. the control (missense) groups of each treatment day. $^{\#}p < 0.05$ vs. the AMPH-treated groups of each treatment day. Bars are the mean ± SEM. $N = 8$ per group.

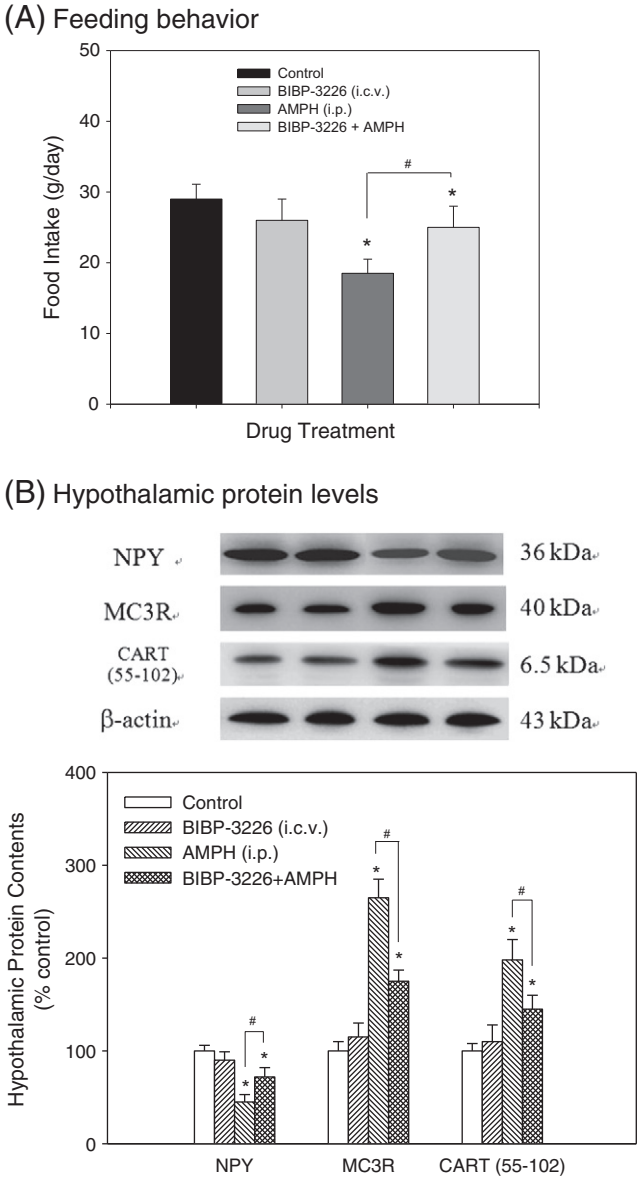

Fig. 5. (A) The effect of BIBP-3226 (Y1R inhibitor) pretreatment on AMPH-induced anorexia during the first day of drug treatment. AMPH-induced anorexia could be modulated by prior administration of BIBP-3226 (80 nmol, i.c.v.). (B) Results showed the relative densitometric values for the changes in hypothalamic protein contents during the first day of 4 mg/kg AMPH treatment. Contents of NPY, MC3R, and CART (55–102) in BIBP- and/or AMPH-treated group were indicated as the percentage of controls. $^{*}p < 0.05$ vs. the control (vehicle-treated, i.c.v.) group. The vehicle is an aCSF solution. $^{\#}p < 0.05$ vs. the vehicle/AMPH-treated groups of each treatment day. Bars are mean ± SEM. N = 6–8 per group.

Effects of pretreatment with NPY antisense on food intake and body weight

Results shown in Fig. 3 revealed that a pretreatment with NPY antisense could modulate the anorectic response of AMPH, indicating the involvement of NPY in AMPH anorexia. The repeated measures of general linear model showed significant differences of the treatments with NPY antisense and combination of NPY antisense and AMPH, compared with the control. There were time effects [$F(4,112) = 21.04$, $p < 0.001$], treatment effects [$F(3,28) = 7.86$, $p < 0.001$], and time-by-treatment interactions [$F(12,112) = 5.13$, $p < 0.001$] (all partial $\eta^2 > 0.36$). Post hoc test by LSD showed lowered food intake in the groups receiving AMPH [$p < 0.05$] and NPY antisense+AMPH [$p < 0.05$], compared with the control group. Furthermore, paired comparisons revealed that reduction of food intake was observed on Days 1–3 after AMPH treatment and on Days 1–4 after NPY antisense+AMPH treatment [all Cohen's $d > 1.15$], compared with Day 0. Moreover, to compare the food intake between antisense/AMPH- and AMPH-treated groups, it revealed a significant difference on Day 3 and Day 4. Taken together, the present results revealed that NPY knockdown might be associated with the enhancement of AMPH-induced anorectic response.

The feeding behavior in missense-treated rats was similar to that in saline-treated rats during a 4-day period of treatment. Moreover, the anorectic response in missense/AMPH-treated rats (Fig. 3) was not significantly changed compared to that in AMPH-treated rats (upper panel of Fig. 1). These results revealed the noninterference of missense treatment in this study.

Results shown in the lower panel of Fig. 6 revealed that a pretreatment with NPY antisense could enhance the decrease of body weight in AMPH-treated rats. The changes in daily body weight during the NPY antisense and/or AMPH treatment were decreased and expressed in a pattern similar to the decrease of daily food intake.

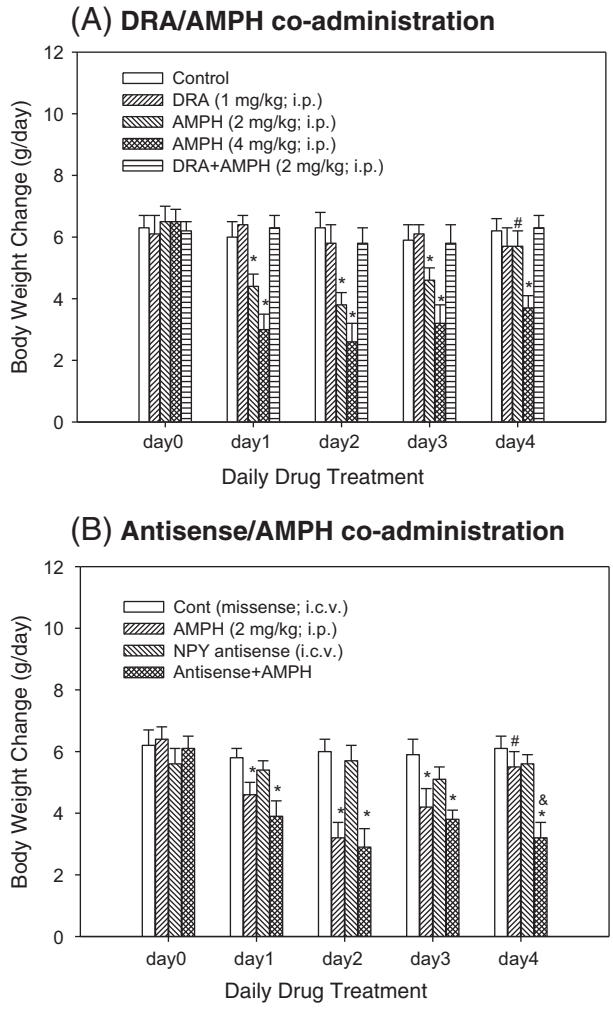

Fig. 6. (A) Effects of daily co-administration with dopamine receptor antagonist (DRA) and AMPH or (B) daily co-administration with NPY antisense and AMPH on the change of body weight during a 4-day period of drug treatment. The change of body weight was calculated with respect to the body weight of the previous day. Each data were expressed as mean ± SEM of eight rats for each group. *Indicates $p < 0.05$ compared to the control group. #Indicates $p < 0.05$ compared to the group on Day 2. &Indicates $p < 0.05$ compared to the AMPH-treated group.

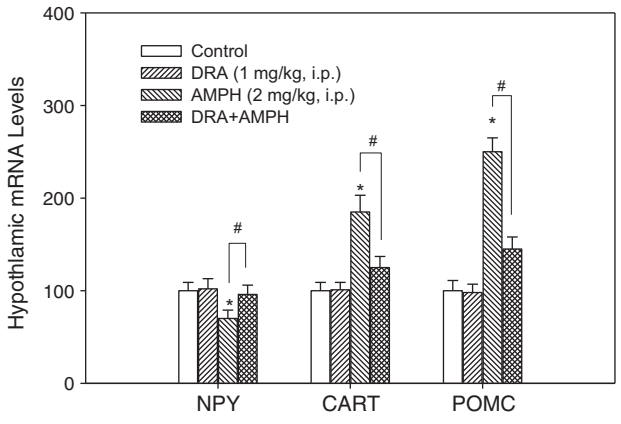

Fig. 7. The effect of dopamine receptor antagonist (DRA) pretreatment on the changes of NPY, CART, and POMC mRNA levels in the hypothalamus in AMPH-treated rats on Day 1. The drug DRA is haloperidol. The mRNA levels in drug-treated groups were indicated as the percentage of the control group (Day 0). Each densitometric value represents the mean ± SEM of 6–8 rats. *$p < 0.05$ vs. the control group. #$p < 0.05$ vs. the AMPH-treated group.

The effect of NPY antisense on NPY, MC3R, and CART expression

As shown in Fig. 4, NPY antisense by itself could reduce NPY but showed no significant effect on CART (55–102) and MC3R expression compared to the control (missense-treated) group, revealing a specific effect of NPY antisense on the decrease of NPY expression. Using β-actin as the internal standard, the ratio of NPY, MC3R, or CART (55–102) over β-actin in each group was calculated and compared. By one-way ANOVA followed by Dunnett's test ($p < 0.05$), it revealed that NPY decreased by approximately 53% in the antisense-treated, 45% in the AMPH-treated, and 40–58% in the antisense/AMPH-treated rats compared to the control group [$F(6,35) = 4.11$, $p < 0.05$, $\eta^2 = 0.31$]. By contrast, CART (55–102) expression increased by approximately 100–200% in both the AMPH-treated and antisense/AMPH-treated groups compared to the control group [$F(6,35) = 8.85$, $p < 0.05$, $\eta^2 = 0.62$]. Similarly, MC3R increased by approximately 100–200% in both AMPH-treated and antisense/AMPH-treated rats compared to the control group [$F(6,35) = 9.68$, $p < 0.05$, $\eta^2 = 0.67$]. Taken together, results revealed that a pretreatment with NPY antisense in the AMPH-treated group resulted in reductions of NPY on Day 3 and Day 4, but resulted in increases of CART (55–102) and MC3R on Day 3 and Day 4, compared to the AMPH-treated group.

Effects of BIBP-3226 on feeding and expression levels in NPY, MC3R, and CART

Results shown in part (A) of Fig. 5 revealed that pretreatment with BIBP-3226 before AMPH could attenuate an AMPH-induced anorectic response. Statistical analysis with one-way ANOVA revealed a significant effect [$F(3,31) = 12.10$, $p < 0.05$, $\eta^2 = 0.56$.]. The LSD post hoc test showed that AMPH decreased food intake [$p < 0.05$, Cohen's $d = 3.11$], compared with controls. Food intake in the group treated with BIBP-3226+AMPH was also lower than that in controls [$p < 0.05$, Cohen's $d = 1.16$] but higher than that in the AMPH-treated group [$p < 0.05$, Cohen's $d = 1.80$]. AMPH could decrease the food intake and pretreatment with BIBP-3226 could reverse food intake by 50% compared to the AMPH-treated group. The food intake in vehicle-treated or aCSF-treated (control) rats was similar to that in saline-treated rats, revealing the non-interference of vehicle in this study. Moreover, the expression of feeding in the BIBP-3226-treated rats was slightly but not significantly reduced (decreased by 10% food intake) compared to that in vehicle-treated rats, revealing that BIBP-3226 had no significant effect on basal food intake in a 24-h testing period.

Results shown in part (B) of Fig. 5 revealed that BIBP-3226 treatment alone didn't affect the expression levels of NPY and MC3R compared to the control group. However, pretreatment with BIBP-3226 in the AMPH-treated rats resulted in partial restorations of NPY, MC3R, and CART (55–102) levels toward normal level. Using β-actin as the internal standard, the protein ratio of NPY, MC4R, or CART (55–102) over β-actin in each group was calculated and compared. The one-way ANOVA [$F(3,31) = 12.10$, $p < 0.05$, $\eta^2 = 0.76$.] followed by LSD post hoc test revealed significant decreases of NPY content in the AMPH-treated [$p < 0.05$, Cohen's $d = 3.86$] and BIBP-3226/AMPH-treated [$p < 0.05$, Cohen's $d = 2.01$] groups, compared to the control group. Moreover, BIBP-3226 could partially restore NPY expression toward normal level, compared to the AMPH-treated group. However, MC3R and CART (55–102) contents were increased in the AMPH-treated and BIBP-3226/AMPH-treated groups (all $p < 0.001$, all Cohen's $d > 2.59$), compared to the control group. Moreover, BIBP-3226 could partially restore MC3R expression about 58% toward normal level compared to the AMPH-treated group.

Discussion

The present study revealed that, in AMPH-treated rats, (1) hypothalamic CART was associated with susceptibility to appetite suppression,

which is controlled by a reciprocal regulation between NPY and POMC neurons; (2) CART was increased and expressed in a manner consistent with that of MC3R (or POMC mRNA), but opposite to the reduction of NPY; (3) in addition to NPY and MC3R, inhibition of Y1R in the brain attenuated the anorectic effect and modified the expression levels in CART; and (4) central dopamine was involved in regulating hypothalamic CART expression. These findings suggest that hypothalamic CART neurons participate in the reciprocal regulation between POMC- and NPY-containing neurons during the regulation of AMPH-induced appetite control.

The current results revealed that daily treatment with AMPH markedly reduced food intake and NPY expression (anorectic effect) on Days 1 and 2, followed by a gradual reversion to normal level from Day 2 to Day 4 (i.e., development of tolerance to AMPH). Thus, NPY reduction participated in the anorectic response of AMPH, while the reversion of NPY back to normal was associated with gradual tolerance to AMPH (Hsieh et al., 2007a, 2007b, 2007c). By contrast, POMC and CART increased during AMPH treatment, with the maximum response on Day 2; this response was opposite to the NPY reduction response, which had its maximum decrease on Day 2. These results implied that POMC- and CART-containing neurons might function together in a manner reciprocal to that of NPY-containing neurons during the regulation of AMPH-evoked appetite suppression. The reason for the reciprocal regulation between NPY and POMC neurons is that NPY can inhibit POMC neurons via the release of GABA (Cowley et al., 2001) or via unidirectional inhibitory input from NPY to POMC neurons (Gong et al., 2008; Hsieh et al., 2007a). Moreover, NPY- and POMC-containing neurons function reciprocally in the regulation of appetite suppression in rats treated with AMPH-like anorectic drugs (Hsieh et al., 2013b). Thus, the reduced inhibition (disinhibition) from NPY to POMC neurons leads to activation of the POMC gene and some cytoplasmic protein kinases in the POMC neuron, such as protein kinase C and protein kinase A, which are activated and expressed in a pattern similar to that of POMC (Hsieh et al., 2007a; Kuo et al., 2011). In the present study, results showed that CART was increased and expressed in a pattern similar to that of POMC; therefore, it is possible that POMC and CART neurons function together in a manner reciprocal to that of NPY neurons during the regulation of AMPH-induced appetite suppression.

To investigate further the role of CART in the reciprocal regulation between NPY- and POMC-mediated appetite controls, the following two experiments were performed. Firstly, using cerebral infusion with NPY antisense, which aims to knockdown NPY expression, we found that pre-treatment with NPY antisense enhanced the reduction of NPY from Day 1 to Day 4. By contrast, NPY knockdown increased the expression of MC3R and CART (55–102) from Day 1 to Day 4, directly opposite to the decrease in NPY expression, in the antisense/AMPH-treated rats compared to the AMPH-treated rats. These results revealed that NPY could inhibit both POMC and CART neurons, which might be due to a unidirectional inhibitory input from NPY to both POMC and CART neurons. Secondly, using cerebral infusion with Y1R inhibitor, we found that pre-treatment with Y1R inhibitor attenuated the decreases in food intake and NPY expression, while it attenuated the increases in MC3R and CART (55–102) expression in AMPH-treated rats. These results revealed that Y1R participated in the regulation of CART-involved appetite control in AMPH-treated rats. Taken together, it is suggested that CART-containing neurons participate in the reciprocal regulation between NPY- and POMC-containing neurons during AMPH treatment.

The current results revealed that CART (55–102), but not CART (62–102), is activated and expressed in a pattern similar to that of MC3R during AMPH treatment, revealing a functional effect of CART (55–102) on AMPH-induced appetite suppression. CART (55–102) and CART (62–102) exhibit different relative activities in different testing paradigms (Vicentic et al., 2006). Most studies have reported that CART (55–102) is more potent than CART (62–102) in the decrease of food intake and body weight gain (Bannon et al., 2001; Vicentic and Jones, 2007). In the present study, CART (62–102) increased gradually from Day 2 to Day 4 during the period of AMPH tolerance, implying that CART (62–102) might play an essential role in the development of tolerance to AMPH anorexia. As described in our previous reports, several other mechanisms, such as the gradual reversion of NPY expression (Hsieh et al., 2007b), the gradual increase of neuronal nitric oxide synthase (Kuo et al., 2010), and a gradual accumulation of delta FosB (unpublished), also responded to daily AMPH treatment during the period from Day 2 to Day 4. These results indicated that CART (62–102) might play a functional role in the induction of AMPH tolerance.

Previous reports discussing the effect of CART on appetite control and energy homeostasis were controvertible. One report indicated that the chronic i.c.v. infusion of CART (55–102) had marked, sustained inhibitory effects on food intake and body weight gain, suggesting that the CART pathway is an important determinant of body weight homeostasis (Rohner-Jeanrenaud et al., 2002). By contrast, administering CART into the paraventricular nucleus (PVN) (Abbott et al., 2001) and using an adenoviral vector to inject CART into the PVN (Smith et al., 2008), both treatments resulted in increased food intake and body weight gain. The reason for these opposite effects of CART is unknown, but it might be related to the behavioral abnormality induced by i.c.v. infusion of CART (Parker and Bloom, 2012). It is also possible that the direct infusion of CART into the PVN using a stainless cannula might mechanically destroy the neural communication between orexigenic NPY and/or anorexigenic CART/POMC, which are in a dynamic and balanced state during the regulation of energy metabolism.

In addition to the modulation of NPY and POMC (MC3R), the present results showed that inhibition of Y1R in the brain also modified the expression levels in CART (55–102). This result revealed that Y1R participated in the reciprocal regulation between NPY- and POMC/CART-containing neurons during AMPH treatment. Consistent with our previous reports indicating that inhibition of Y1R can modify the expression levels of hypothalamic NPY and POMC during AMPH treatment (Kuo et al., 2012a), the present results suggest that NPY-Y1R neurotransmission is also involved in regulating CART-mediated appetite control.

The present results showed that central dopamine was involved in regulating hypothalamic CART expression during AMPH treatment. It is because pretreatment with DRA, i.e. haloperidol, before AMPH treatment modified the anorectic effect of AMPH and changed the expression levels in hypothalamic NPY, POMC, and CART. Consistent with our previous report, it revealed that both AMPH-induced anorexia and NPY decrease are dependent on the central effect of dopamine, but are independent of the peripheral factors, such as blood insulin, glucocorticoid, and leptin, as these factors remain unchanged during daily AMPH treatment (Kuo and Cheng, 2002). Here, we expanded our findings that central effect of dopamine during AMPH treatment could also increase hypothalamic POMC, and CART expression. Compared to the previous report indicating the central effect of leptin, which acts as a negative feedback signal to the brain in appetite control through the stimulation of CART peptide and the inhibition of NPY secretion (Kristensen et al., 1998; Lambert et al., 1998), the present results revealed that central dopamine also showed a similar negative feedback signal mechanism in appetite control in AMPH-treated rats.

In the present study, we might well believe that it was a suitable time to detect the gene expression of NPY, POMC, and CART during the initial 40–60 min after AMPH treatment. Our previous reports revealed that (1) AMPH treatment can decrease food intake and modulate hypothalamic NPY and POMC expression levels during this period of time (Hsieh et al., 2007c, 2011); (2) several transcription factors, such as AP-1 (Hsieh et al., 2006), CREB (Hsieh et al., 2007c), NF-κB (Kuo et al., 2012b), and STAT3 (Chu et al., 2014), are activated and involved in regulating NPY gene expression during this period; and (3) from the time courses of a 24-h feeding behavior following a single dose of AMPH treatment, result reveals that the anorectic response happens only at the initial 0–6 h time interval,

but not the other time intervals (6–12 h, 12–18 h, and 18–24 h) (Kuo, 2005).

Drugs targeting NPY, CART, or POMC expression have been suggested as the clinical pharmacotherapy in the treatment of obesity (Aronne and Thornton-Jones, 2007). Based on previous findings that CART is co-localized with the hypothalamic POMC neurons (Vrang et al., 1999), that obese animals have little or no expression of CART, and that food restriction reduces CART levels in the hypothalamus of lean animals (Kristensen et al., 1998), CART has been added to the list of feeding-related neuropeptides under the current rationale and is regarded as an endogenous satiety factor (Parker and Bloom, 2012).

In conclusion, the present results suggest that cerebral dopamine is involved in regulating hypothalamic CART expression, which participates in the reciprocal regulation between NPY and POMC neurons during the control of AMPH-induced appetite suppression. Moreover, dopamine–NPY-Y1R neurotransmission in the brain participates in regulating hypothalamic CART-mediated appetite control in AMPH-treated rats.

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科技部補助計畫衍生研發成果推廣資料表

日期:2015/09/17

科技部補助計畫	計畫名稱: 厭食劑在腦內之分子機制：NPY受器、GRE-DNA結合位及STAT3轉錄因子之角色
	計畫主持人: 郭東益
	計畫編號: 101-2320-B-040-002-MY3　　學門領域: 生理

無研發成果推廣資料

101 年度專題研究計畫研究成果彙整表

計畫主持人：郭東益	計畫編號：101-2320-B-040-002-MY3
計畫名稱：厭食劑在腦內之分子機制：NPY 受器、GRE-DNA 結合位及 STAT3 轉錄因子之角色	

成果項目			量化			單位	備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等）
			實際已達成數（被接受或已發表）	預期總達成數(含實際已達成數)	本計畫實際貢獻百分比		
國內	論文著作	期刊論文	1	1	100%	篇	於國內中山醫學雜誌發表
		研究報告/技術報告	0	0	100%		
		研討會論文	1	1	100%		於生物醫學年會發表
		專書	0	0	100%		
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（本國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		
國外	論文著作	期刊論文	2	2	100%	篇	於外國雜誌發表 Archives Toxicology ; British Journal of Pharmacology
		研究報告/技術報告	0	0	100%		
		研討會論文	2	2	100%		於英國利物浦及新加坡研討會發表
		專書	0	0	100%	章/本	
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（外國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		

其他成果 (無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。)	無

	成果項目	量化	名稱或內容性質簡述
科教處計畫加填項目	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	
	計畫成果推廣之參與（閱聽）人數	0	

科技部補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估

■達成目標

□未達成目標（請說明，以 100 字為限）

□實驗失敗

□因故實驗中斷

□其他原因

說明：

2. 研究成果在學術期刊發表或申請專利等情形：

論文：■已發表 □未發表之文稿 □撰寫中 □無

專利：□已獲得 □申請中 ■無

技轉：□已技轉 □洽談中 ■無

其他：(以 100 字為限)

研究成果在學術期刊發表:

Kuo DY*, Chen PN, Hsieh YS*. (2015). Targeting oxidative stress in the hypothalamus: the effect of transcription factor STAT3 knockdown on endogenous antioxidants-mediated appetite control. 2015 Jen, 89: 87-100. Archives Toxicology.

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）(以 500 字為限)

了解 Y1R 及 Y5R 受器對 NPY、POMC 神經路徑之調控方式，及 nGRE signaling、STAT3 signaling 調控 POMC 基因表現的分子機制，有助於了解安非他命、搖頭丸等藥物毒性及許多神經病變的形成及治療。神經受傷時，生長因子、激素、及細胞激素均透過 STAT3 來執行它們的神經保護作用及神經再生，因此，控制 STAT3 可用於治療急性神經受傷或慢性神經再生有關疾病上