Both CART and POMC Neurotransmission Participate in Neuropeptide Y-mediated Appetite Control in Rats Treated with Amphetamine

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Hypothalamic neuropeptide Y (NPY) and proopiomelanocortin (POMC) are involved in the regulation of amphetamine (AMPH)-induced appetite suppression. The aim of this study was to examine whether hypothalamic cocaine- and amphetamine-regulated transcript (CART) is also involved in the acute anorectic action of AMPH. Rats were treated with AMPH and changes in feeding behavior and expression levels of NPY, POMC, and CART were assessed and compared 24 h later. Results showed that feeding and expression level of NPY decreased, while the expressions of CART (55-102), melanocortin 3 receptor (MC3R), and MC4R increased, following AMPH treatment. To examine the role of NPY in regulating POMC and CART, intracerebroventricular infusion of NPY antisense was applied 60 min prior to AMPH treatment. NPY knockdown led to further decreases in AMPH-induced anorexia and NPY expression and increases in MC4R, MC3R, and CART (55-102) expressions. This indicated that both hypothalamic CART and POMC participate in the regulation of NPY-mediated appetite control during AMPH treatment. The results of this study may advance the knowledge regarding the molecular mechanism of AMPH-induced appetite suppression.

Keywords: CART, POMC, NPY, amphetamine, appetite, hypothalamus

Introduction

The mechanism underlying the appetite-suppressing effect of amphetamine (AMPH) is associated with the central release of dopamine, which decreases neuropeptide Y (NPY) but increases proopiomelanocortin (POMC) in the hypothalamus¹. Moreover, it has been reported that orexigenic NPY and anorexigenic POMC peptides function reciprocally in the regulation of appetite suppression in rats treated with AMPH or AMPH-like anorectic drugs¹. Although NPY functions in a manner opposite to that of POMC during the control of energy balance, it is unknown whether the cocaine- and amphetamine-regulated transcript (CART) system, another anorexigenic drive, is involved in regulating NPY-mediated appetite suppression in AMPH-treated rats.

Hypothalamic NPY is a highly conserved neuropeptide that regulates several physiological responses, such as feeding behavior¹, energy balance¹, processing of emotions⁹, and prevention of oxidative stress¹⁰. Electrophysiological evidence has revealed that 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\[^{[11]}\].

CART was initially identified as a result of its positive regulation by the psychomotor stimulants cocaine and amphetamine\[^{[12]}\]. The colocalization of CART with both orexigenic NPY and anorexigenic neuropeptides POMC suggests that CART has a modulatory role in feeding behavior\[^{[13-15]}\]. Moreover, the CART peptide is a modulator of dopamine and psychostimulants. CART tends to oppose large increases in dopamine signaling\[^{[16]}\] and prevent oxidative stress in the brain\[^{[17]}\]. Thus, it is possible that CART participates in the regulation of 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\[^{[18]}\]. CART (55–102) is five-fold more potent than CART (62–102) in the inhibition of food intake\[^{[19]}\]. Thus, we hypothesized that hypothalamic CART is involved in the reciprocal regulation between NPY and melanocortin 3 receptor (MC3R), as well as between NPY and melanocortin 4 receptor (MC4R). MC3R and MC4R are two members of the POMC system involved in the control of AMPH-induced anorexia.

### 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 habituated to frequent handling. Drugs were administered and food intake was determined daily at the beginning of the dark phase (6:00 p.m.). Water and chow (LabDiet) were freely available throughout the experimental period. Food intake data points above 35 g/day were discarded as they indicated food spillage. All of the procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals adopted by the National Institutes of Health.

#### Drugs, Chemicals, and Reagents

Chow (LabDiet) was purchased from PMI Nutrition International (Brentwood, MO, USA). AMPH, angiotensin II, and Tris-HCl were obtained from Sigma-Aldrich (St. Louis, MO, USA). Antibodies against NPY and β-actin were supplied by Santa Cruz Biotechnology (Santa Cruz, CA, USA). MC3R and MC4R antibodies were purchased from Gibco BRL, Life Technologies, Inc. (Rockville, MD, USA), while CART (55-102) and CART (62-102) antibodies were supplied by Phoenix Pharmaceuticals, Inc. (Burlingame, CA, USA). Semi-quantitative RT-PCR analysis was performed using 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 effects 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 1, 2 or 4 mg/kg for 1 day. AMPH was first injected at the end of Day 0 (i.e. at 6:00 p.m. or at the beginning of Day 1). Changes in food intake and body weight were calculated with respect to the amount of food consumed and the body weight on the previous day.

To determine the acute effects of AMPH treatment (2 mg/kg; i.p.) on the changes in hypothalamic NPY, CART, and POMC mRNA levels, rats were injected with the drug for 1 day. Similarly, to determine the acute effects of AMPH (2 mg/kg; i.p.) on the changes in hypothalamic NPY, CART (55-102), CART (62-102), MC3R, and MC4R expressions, rats were injected with AMPH for 1 day. On the sacrifice day, rats received 2 mg/kg AMPH 40 min before being sacrificed to enhance the effects of AMPH. The rats were anesthetized with 35 mg/kg pentobarbital and decapitated. Following decapitation, the hypothalamus was immediately removed to
determine mRNA levels or protein expressions or stored at –80 °C until analysis.

To assess the effects of pretreatment with NPY antisense oligodeoxynucleotide on the anorectic response of AMPH, rats (N=8 per group) were administered NPY antisense (20 μg in a 10-μl vehicle) via intracerebroventricular (i.c.v.) injection 1 h before AMPH (4 mg/kg; i.p.) treatment. Prior to this, rats were administered a similar dose of NPY antisense via i.c.v. injection daily for 2-3 days until the feeding behavior was slightly reduced, as continuous or repeated i.c.v. injections of antisense may be necessary to maximize behavioral effects and to block the synthesis of a constitutively active gene product[20,21]. Descriptions of the surgery for i.c.v. cannulation and antisense are described in the relevant section.

To examine the effects of NPY antisense (or missense) on NPY, MC3R, MC4R, and CART (52-102) expressions, rats (N=6-8) were infused with antisense or missense (20 μg in a 10-μl vehicle; i.c.v.) 1 h before 2 mg/kg AMPH treatment. Prior to this, rats were i.c.v. infused with similar dose of antisense (or missense) daily for 2-3 days until the feeding behavior was slightly reduced in the antisense group. At 40 min after antisense (missense) and/or AMPH treatment, rats were anesthetized and the hypothalamus was removed. NPY, MC3R, MC4R, and CART (52-102) expression levels were determined on Western Blot.

**RNA Extraction**

Hypothalamic NPY, POMC, and CART mRNA levels were measured in a block of mediobasal hypothalamic tissue as previously described [22]. In brief, total RNA was isolated from this block using the modified guanidinium thiocyanate-phenol-chloroform method [23]. 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 were added to each sample followed by vigorous shaking for 15 sec, incubation at 22 °C for 3 min and centrifugation at 12,000 ×g at 4 °C for 15 min. 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 at 4 °C for 15 min. The gel-like RNA pellets were washed with 75% ethanol by vortexing followed by centrifugation at 7,500 ×g at 4 °C for 5 min. Thereafter, RNA pellets were briefly dried, dissolved in RNase-free water, and stored at -80 °C. The content of RNA was determined spectrophotometrically at 260 nm (Hitachi U-3210, Japan).

**Reverse Transcription-Polymerase Chain Reaction (RT-PCR)**

Using the 1st Strand cDNA Synthesis Kit (Boehringer Mannheim GmbH, Germany), RNA was reversely transcribed into single-stranded cDNA. For each sample, 8 μl of sterile DEPC (diethyl pyrocarbonate) water containing 2 μg of RNA were added to oligo-p(dT)15 primer (0.8 μg/μl), followed by heating at 65 °C for 15 min, cooling at 25 °C for 10 min. This was then combined with reaction mixture consisting of 10x reaction buffer (100 mM Tris, 500 mM KCl; pH 8.3), deoxynucleotide mix (10 mM each), MgCl2 (25 mM), RNase inhibitor (40 unit/μl), and AMV reverse transcriptase (25 unit/μl). Reaction mixtures were incubated at 42 °C for 2 h and then brought to 95 °C for 5 min to terminate the reaction, followed by soaking at 16 °C. PCR was subsequently carried out by mixing 3 μl of cDNA product with mastermix solution consisting of DEPC water, 10x reaction buffer, MgCl2 (25 mM), deoxynucleotide mix (10 mM each), P1 and P2 primers (1 μg/μl each), and Taq polymerase (5 unit/μl). GAPDH was used as the internal standard calibrator. PCR reactions for NPY were carried out on a PCR thermocycler (Perkin-Elmer GeneAmp 2400) for 28 cycles with each cycle consisting of the following steps: 91 °C for 1 min (denaturing), 60 °C for 1 min (annealing), and 72 °C for 30 sec (extension), followed by a final elongation step at 72 °C for 7 min. Finally, the PCR products were soaked at 16 °C. PCR reactions for the remaining molecules were carried out as described above except for changes in the annealing temperatures and numbers of cycles as follows: POMC (55 °C, 25 cycles); CART (60 °C, 28 cycles ); GAPDH (52 °C, 25 cycles). The sequences of primers used in RT-
Gel Electrophoresis

At the completion of RT-PCR, 8 μl of each PCR product were separated by flat-bed gel electrophoresis onto a 3% agarose gel. Gels stained with ethidium bromide (0.5 μg/ml, Sigma-Aldrich Co., MO, USA) were visualized under UV light, photographed, and densitometrically scanned. Ratios of NPY and GAPDH mRNA were calculated to determine relative NPY mRNA levels. Contents of NPY mRNA in AMPH-treated group were indicated as the percentage of control group. The ratio of NPY/GAPDH mRNA was measured by digital densitometry (Hoefer, San Francisco, CA, USA). Similar steps were used to determine POMC and CART mRNA levels.

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). 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, cannula placement was verified by histochemistry of brain section and by the administration of angiotensin II (100 ng/rat). Angiotensin II reliably induces water drinking in non-deprived rats when administered into the cerebroventricles. 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. Then, angiotensin II was administered to confirm the cannula placement and restoration of normal drinking behavior. Two days later, AMPH treatment was started (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 access no. 15880) was selected. The antisense ODN (5’-CCCCATTCGTTTGTACC) was inversely complementary to this sequence. Phosphorothioate internucleotide linkages were obtained by treatment with tetraethylthiuram disulfide and the resulting phosphorothioate ODNs were purified and lyophilized. An 18-mer missense ODN (5’-TTATTCCCCAGTTTGC) served as the control. As previously described, the antisense sequence did not display self-hybridization; therefore it was effective in blocking the message read-through. In addition, one week of daily i.c.v. injections of the antisense reduced food intake and body weight when compared with the missense-treated control. Rats were handled to carry out i.c.v. injection 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/10μl/day) was administered to 8 rats, and the same treatment

Table 1. Sequences of primers used in RT-PCR.

<table>
<thead>
<tr>
<th>Genes</th>
<th>GenBank accession No.</th>
<th>Primer</th>
<th>Sequence 5’→3’</th>
<th>Size of product (base pairs)</th>
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<tr>
<td>NPY</td>
<td>NM_012614</td>
<td>Forward GGGCTGTGTGGACTGACC</td>
<td>264</td>
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<tr>
<td></td>
<td></td>
<td>Reverse GGAAGGTCTTCAAGCCT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>POMC</td>
<td>NM_139326</td>
<td>Forward GAGATTCTGCTACAGTCGCT</td>
<td>678</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse TTGATGATGCGTCTTGGAA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CART</td>
<td>NM_017110.1</td>
<td>Forward CTCTGGGGCGCCCGCCTGCTG</td>
<td>252</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse CATGGGGACTTGGCCGTACTTC</td>
<td>213</td>
<td></td>
</tr>
<tr>
<td>GAPDH</td>
<td>NM_017008</td>
<td>Forward TCCCTCAAGATTTCTCAGCAA</td>
<td>309</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Reverse AGATCCACAACGGGATACATT</td>
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was repeated for 7 days. An equivalent dose of missense ODN was administered to each of the 8 rats to serve as the control. Food intake and body weight changes were recorded daily. NPY antisense was phosphorothioate-modified (S-ODNs) only on the three terminal bases at both the 5' and 3' ends, because these S-ODNs improve hybridization affinity and nuclease resistance and are well-established agents in several vertebrate systems \(^2\) and rat brain \(^2\). 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\(_2\)HPO\(_4\) and 0.3 mM NaH\(_2\)PO\(_4\); pH 7.4.

**Western Blotting**

Hypothalamus tissue extracts were subjected to electrophoresis. Proteins were separated on a 12.5% polyacrylamide gel, transferred onto a nitrocellulose membrane, and incubated with specific antibodies against NPY, CART (55-102), CART (62-102), MC3R, MC4R, and β-actin. 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**

Data are presented as mean ± SEM. Two-way or one-way ANOVA followed by Dunnett’s test was used to detect significant differences between groups. Statistical significance was set at \(p < 0.05\).

**Results**

**The effects of AMPH on feeding behavior and body weight**

Changes in 24-h feeding behavior in rats receiving AMPH are shown in the upper panel of Figure 1. One-way ANOVA followed by Dunnett’s test (\(p<0.05\)), revealed a significant dose-dependent effect \([F(4,35)=4.11]\). Moreover, 2 and 4 mg/kg AMPH led to reduced food intake compared to the control.

Changes in 24-h body weight in rats receiving AMPH treatment are shown in lower panel of Figure 1. One-way ANOVA followed by Dunnett’s test \((p<0.05)\), revealed a significant dose-dependent effect \([F(4,35)=3.85]\). Moreover, 2 and 4 mg/kg AMPH reduced the body weight compared to the control. These results revealed that the pattern of decreases in body weight in AMPH-treated rats is similar to that of food intake over a 24-hour period.

**The effects of AMPH on NPY, POMC, and CART mRNA levels**

The ratios of NPY, POMC, and CART mRNA to GAPDH were calculated and compared. From Figure 2, AMPH treatment decreased the expression of NPY but increased the expressions of POMC and CART mRNA, compared to the control, over a 24-h period of AMPH treatment. The effects of AMPH on NPY, MC3R,
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Using β-actin as the internal standard, the ratios of NPY, MC3R, MC4R, CART (55-102), and CART (62-102) to β-actin were calculated and compared. From Figure 3, AMPH treatment decreased NPY but increased MC3R, MC4R, and CART (55-102) expressions compared to the control \([t-test, p<0.05]\). However, CART (62-102) expression remained unchanged over the 24-h period of AMPH treatment.

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

From Figure 4, pretreatment with NPY antisense modulated the anorectic response and body weight change induced by AMPH over a 24-h period, indicating the involvement of NPY in AMPH anorexia. Using one-way ANOVA to analyze the effects of NPY antisense on AMPH anorexia revealed significant treatment-dependent effects \([F(3,28)=4.41]\). Significant differences were observed among antisense-treated, AMPH-treated, and antisense/AMPH-treated groups compared to missense-treated (control) groups. Moreover, there were significant differences in food intake between antisense/AMPH- and AMPH-treated groups, indicating NPY antisense enhancement of AMPH-induced anorexia. Taken together, these results indicated that NPY knockdown enhances the anorectic response of AMPH.

The feeding behavior and body weight changes in missense-treated rats were similar to those in saline-treated rats over a 24-h period. Moreover, the anorectic response and body weight changes in missense/AMPH-treated rats (Figure 4) showed no
significant change when compared with AMPH-treated rats (Figure 1). These results revealed the noninterference of missense treatment in this study.

The effects of NPY antisense on NPY, MC3R, MC4R, and CART expressions

From Figure 5, NPY antisense by itself reduced NPY but showed no significant effects on CART (55-102), MC3R, or MC4R expressions compared to the control (missense-treated) group. This revealed a specific effect of NPY antisense on the decrease in NPY expression. Using β-actin as the internal standard, the ratios of NPY, MC3R, MC4R, and CART (55-102) to β-actin were calculated and compared. One-way ANOVA followed by Dunnett’s test (p<0.05) revealed that NPY decreases by approximately 48% in antisense-treated, 55% in AMPH-treated, and 50% in antisense/AMPH-treated rats compared to the control group [F(3,28)=4.56]. In contrast, CART (55-102) expression increased by approximately 200% in both AMPH-treated and antisense/AMPH-treated groups compared to the control group [F(3,28)=4.05]. Similarly, MC3R increased by approximately 200-230% in AMPH-treated and antisense/AMPH-treated rats compared to the control group [F(3,28)=3.68]. MC4R increased by approximately 180-200% in AMPH-treated and antisense/AMPH-treated rats compared to
the control group \([F(3,28)=3.21]\). Taken together, the results revealed that pretreatment with NPY antisense in AMPH-treated group leads to reduction in NPY, but increases in CART (55-102), MC3R, and MC4R, compared to the AMPH-treated group.

**Discussion**

Results of this study revealed that food intake and body weight dose-dependently decrease following AMPH treatment. Moreover, hypothalamic NPY gene was down-regulated, but POMC and CART genes were up-regulated, during the regulation of AMPH-induced appetite control. NPY knockdown enhanced the decreases in food intake, body weight, and NPY expression and the increases in CART (55-102), MC3R, and MC4R expressions after 24 h drug treatment. These results suggested that both hypothalamic CART and POMC neurotransmission participate in the regulation of NPY-mediated appetite control in AMPH-treated rats.

The present results also revealed that NPY reduction participates in the anorectic response of AMPH. However, POMC and CART increased during AMPH treatment. These results implied that POMC and CART neurons function together in a manner reciprocal to that of NPY neurons during AMPH-induced appetite suppression. Numerous studies have revealed possible roles of NPY, POMC, and CART: (1) There are functional interactions between CART and NPY in the hypothalamus, as neural varicosities containing NPY form dense pericellular baskets around CART-immunoreactive cell bodies\[^{28}\]. (2) NPY can inhibit POMC neurons via the release of GABA\[^{29}\] or via unidirectional inhibitory input from NPY to POMC neurons\[^{30,31}\]. (3) NPY and POMC function reciprocally in the regulation of appetite suppression in rats treated with phenylpropanolamine, an AMPH-like anorectic drug\[^{33}\]. Moreover, consistent with the present findings, previous evidence has revealed that food deprivation suppresses hypothalamic CART and POMC expressions, but increases NPY expression\[^{32}\], and that glucocorticoids are required for meal-induced changes in NPY, CART, and POMC mRNA expressions in the hypothalamic arcuate nucleus\[^{33}\]. The results of the present study provided evidence that the decrease in NPY and the increases in CART and POMC occur together during AMPH treatment.

To further examine the possible roles of POMC and CART in the reciprocal regulation of NPY-mediated appetite control, cerebral infusion with NPY antisense ODN was carried out to inhibit NPY mRNA expression. NPY knockdown in AMPH-treated rats enhanced NPY reduction. In contrast, NPY knockdown increased the expressions of MC3R, MC4R, and CART (55-102) in antisense/AMPH-treated rats compared to AMPH-treated rats. These results revealed that NPY knockdown modulates both POMC and CART expressions, possibly due to unidirectional inhibitory input from NPY to POMC and CART neurons. Thus, it is suggested that both POMC and CART participate in an opposing pattern of regulation of NPY during AMPH treatment.

The present results showed that CART (55-102), but not CART (62-102), is activated during AMPH treatment, revealing a functional effect of CART (55-102) on AMPH-induced appetite suppression. Previous evidence has revealed that CART (55-102) and CART (62-102) exhibit different relative activities in different testing paradigms\[^{34}\], and that CART (55-102) is more potent than CART (62-102) in decreasing food intake and body weight\[^{34,35}\].

Previous reports have discussed the effects of CART on appetite control and energy homeostasis with controversial results. One such report indicated that chronic i.c.v. infusion of CART (55-102) has an inhibitory effect on food intake and body weight gain, suggesting that the CART pathway is an important determinant of body weight homeostasis\[^{36}\]. In contrast, administering CART into the paraventricular nucleus in the hypothalamus\[^{37,38}\] may result in increased food intake and body weight gain. The reason for the opposing effects of CART is unknown, but it might be related to the complex behavioral abnormality induced by i.c.v. infusion of CART\[^{39}\]. It is also possible that the direct infusion of CART into the paraventricular nucleus, using a stainless cannula, mechanically destroys the neurotransmission
of orexigenic NPY and/or anorexigenic CART/ POMC, leading to a metabolic imbalance.

In the present study, we used NPY antisense, but not CART inhibitor, to investigate the role of CART in regulating NPY-mediated appetite control, as no CART receptors have been identified due to the various physiological functions of CART\(^{[39]}\), such as endocrine control\(^{[40,41]}\), drug-related reward and addiction\(^{[34]}\), and anxiety control\(^{[42]}\). Thus, the mechanisms by which CART affects food intake are poorly understood\(^{[19]}\). However, the consistent changes in CART and POMC (including MC3R and MC4R) expressions during AMPH administration or NPY antisense/AMPH co-administration reveal that both POMC and CART (55-102) participate in NPY-mediated appetite control in AMPH-treated rats.

Clinical drugs targeting the expressions of hypothalamic neuropeptides, including NPY, CART, and POMC, have been suggested in the treatment of obesity\(^{[43]}\). Based on previous findings that CART is colocalized with hypothalamic POMC neurons\(^{[44]}\) and that food restriction reduces POMC and CART levels in the hypothalamus in lean animals\(^{[45]}\), CART and POMC are regarded as endogenous satiety neuropeptides\(^{[39]}\).

In conclusion, the present results suggest that both hypothalamic POMC and CART neurotransmissions participate in the regulation of NPY-mediated appetite control in AMPH-treated rats.

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