Available online at www.sciencedirect.com
SCIENCE DIRECT®
Regulatory Peptides 115 (2003) 73–80
REGULATORY PEPTIDES
www.elsevier.com/locate/regpep

# Systemic 5-hydroxy-L-tryptophan down-regulates the arcuate CART mRNA level in rats

Si Ho Choi[a], Bum Sup Kwon[b], Seoul Lee[a], Thomas A. Houpt[c], Hoon Taek Lee[b], Dong Goo Kim[a], Jeong Won Jahng[a,*]

[a] *Department of Pharmacology and Yonsei Brain Research Institute, BK21 Project for Medical Science, Yonsei University College of Medicine, Seoul 120-752, South Korea*
[b] *Department of Animal Science, Konkuk University College of Agriculture, Animal and Life Science, Seoul 143-701, South Korea*
[c] *Department of Biological Science, Florida State University, Tallahassee, FL 32306, USA*

Received 12 August 2002; received in revised form 15 April 2003; accepted 22 April 2003

**Abstract**

This study was conducted to determine if serotonin (5-hydroxytryptamine; 5-HT) system correlates with the hypothalamic expression of cocaine-amphetamine-regulated transcript (CART) gene. Rats received intraperitoneal 5-hydroxy-L-tryptophan (5-HTP; a single or three daily injections at a dose of 100 mg/kg/10 ml), and CART mRNA level in the hypothalamus was examined by in situ hybridization at different time points. The 5-HT contents of the hypothalamus as well as the brainstem was increased persistently by 5-HTP injections, and food intake and body weight gain reduced. CART mRNA level decreased significantly in the hypothalamic arcuate nucleus by three daily 5-HTP, but not by a single injection. The pair-fed group of the chronic 5-HTP did not show a decrease in the arcuate CART mRNA level. The plasma leptin level markedly decreased in the chronic 5-HTP group, compared to the saline group, however, still higher than the pair-fed group with a statistical significance. These results suggest that 5-HT may suppress CART mRNA expression in the arcuate nucleus, not only by leptin signaling via its anorectic effect on the control of food intake, but also by some non-leptin mediated pathway.


*Keywords:* Gene expression; Hypothalamus; Serotonin; Leptin

## 1. Introduction

Cocaine-amphetamine-regulated transcript (CART) was firstly identified in the rat brain by PCR differential display after the acute administration of psychomotor stimulants [1]. CART mRNA expression is widely detected in the central nervous system, massively in the hypothalamic areas such as the paraventricular nucleus (PVN), arcuate nucleus (ARC), dorsomedial hypothalamus (DMH) and lateral hypothalamus (LHA) [2,3]. The hypothalamic CART has been reported to play a role in the central control of energy homeostasis as an anorectic molecule, and its expression is regulated by plasma leptin [4,5]. It has recently been reported that the rats received intracerebroventricular administration of CART (55–102) peptides showed behavioral change resembling the typical behavioral symptoms found in the mice carrying disorders in the brain serotonergic system [6]. This finding suggests that CART may have a functional correlation with serotonin (5-hydroxytryptamine; 5-HT) in the regulation of central nervous system. It was reported that CART (55–102) peptides showed no effect on 5-HT release from the hypothalamic synaptosomes in vitro system [7], however, it has not yet been reported whether there is a molecular interaction between the central 5-HT and the hypothalamic CART in vivo system.

5-HT is known to play a role in feeding behavior as an anorectic molecule [8], has been implicated in the processes of within-meal satiation and post-meal satiety [9]. It was reported that fluoxentine, a selective serotonin reuptake inhibitor, suppresses food intake and body weight gain, whereas metergoline, a 5-HT1/5-HT2 receptor antagonist, as well as 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), a 5-$HT_{1A}$ receptor agonist, enhances food consumption [10,11]. 5-$HT_{1A}$ receptor immunoreactivity is observed in the hypothalamic arcuate neurons containing CART, neuropeptide Y (NPY), agouti-related peptide, or proopio-

* Corresponding author. Tel.: +82-2-361-5233; fax: +82-2-313-1894.
*E-mail address:* jwjahng@yumc.yonsei.ac.kr (J.W. Jahng).

0167-0115/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0167-0115(03)00126-5

melanocortin [12]. The hypothalamus appears to be where 5-HT exerts its anorectic effect in the central control of feeding, perhaps, at least partly, through its interaction with the hypothalamic feeding peptides. It has been reported that 5-HT exhibits a negative correlation with NPY, a potent orexigenic molecule, in the hypothalamus [13–16]. Interestingly, there have been some reports showing an interaction between NPY and CART, such as, intracerebroventricular administration of CART peptides suppresses the NPY effect on food intake in a dose-dependent manner [4,5]. These suggest that both 5-HT and CART may exert their effects through the action of NPY as a part of common output for the expression of satiety. However, the hypothalamic expression of CART as well as NPY was significantly decreased in anorectic (*anx*/*anx*) mouse showing drastic activation of the central 5-HT system [15,17,18]. This suggests a possible negative interaction of the central 5-HT with CART expression as it does with NPY.

In order to determine whether the increased activity of the brain 5-HT system negatively interacts with the hypothalamic CART expression, a 5-HT precursor, 5-hydroxy-L-tryptophan (5-HTP), was administrated to rats to increase the brain 5-HT level [19,20]. The CART mRNA level in the hypothalamus was then examined by in situ hybridization technique. The plasma leptin level was also analyzed to determine whether or not the modulation of CART gene expression is an anorectic consequence of the 5-HTP administration, possibly through the leptin-mediated pathway.

## 2. Materials and methods

### 2.1. Animals

Adult male Sprague–Dawley rats (250–300 g, Daehanbiolink, Korea) were individually acclimated to the standard laboratory conditions (12 h light–dark cycle, light on at 9:00 AM) with free access to standard laboratory food (Purina Rodent Chow, Purina, Seoul, Korea) and water ad libitum. Animals were cared according to The Guideline for Animal Experiments, 2000, edited by The Korean Academy of Medical Sciences, which is consistent with NIH Guideline for the Care and Use of Laboratory Animals, 1996 revised.

### 2.2. Drug treatments

Rats were divided into three treatment groups ($n=6$ per group), such as the free fed saline, the 5-hydroxy-L-tryptophan (5-HTP; 100 mg/kg/10 ml, dissolved in sterile physiologic saline; Sigma, MO, USA) injected or the pair-fed group. A single peritoneal injection of 5-HTP was given 1 h before the lights off, or three daily injection at 9:00 AM every morning, respectively. The same volume of saline was injected to the control group for each 5-HTP injection. Body weight and the amount of food intake were recorded during the treatment. Each rat in the pair-fed group was provided with the same amount of food that the 5-HTP rats had consumed. Rats were sacrificed at 2 or 8 h after the single injection of 5-HTP, and 24 h after the last injection of daily 5-HTP or the end of pair-fed ($n=6$).

The injection dose of 5-HTP (100 mg/kg, i.p.) was determined according to previous report [19, 21]. Either a behavioral toxic effect, or the 5-HT syndrome, which occurs by a higher dose of 5-HTP (300 mg/kg) [22] or by a combined treatment of 5-HTP and clorgyline [23–25], was not detected at this dose of 5-HTP.

### 2.3. 5-HT level in each brain region

The tissue samples of the hypothalamus and the dorsal raphe nucleus were dissected out on ice immediately after the decapitation, rapidly frozen with dry ice and stored at −80 °C until used. 5-HT contents of the tissue samples were measured by high-performance liquid chromatography (Waters Instrument, Model 700, Milford, MA, USA) equipped with an ESA Coulochem II Elctrochemical Dector (ESA, Chelmsford, MA, USA) packed by biophase ODS 5 μm (250 × 4.5 mm, Bioanalytical System, West Lafayette, IN, USA) according to a slight modification of the method previously reported [26]. The mobile phase, comprising of acetonitrile 8% and 92% 0.15 M monochloroacetic acid buffer (0.55 mM sodium octyl sulfate, 2 mM disodium EDTA, pH 3.35) was pumped at a rate of 1 ml/min.

### 2.4. Plasma leptin level

The cardiac blood was collected into the heparinized tubes, rapidly after exposing the heart with an overdose of sodium pentobarbital, and centrifuged at 2000 rpm for 20 min to separate the plasma. The plasma leptin level was determined by radioimmunoassay method using a commercial kit (Mediagnost mouse/leptin kit, Aspenhaustr, Reutlingen, Germany).

### 2.5. In situ hybridization

Immediately after collecting the blood, transcardiac perfusion was performed with heparinized isotonic saline containing 0.5% $NaNO_2$, then with 4% paraformaldehyde in 0.1 M sodium phosphate buffer. The brains were rapidly dissected, blocked, post-fixed for 3 h, and transferred into 30% sucrose for 24 h for cryoprotection. Forty-micron coronal sections were cut on a freezing sliding microtome. Every third sections through the rostral-caudal extent of the hypothalamus (between bregma −1.40 and −3.80 mm) and the alternate sections of the raphe nucleus (between bregma −7.64 and −8.80 mm) were collected into 20 ml glass scintillation vials containing ice-cold 2 × SSC (0.3 M NaCl, 0.03 M Na Citrate) for in situ hybridization. All coordinates were based on Paxinos and Watson [27]. The

SSC was pipetted off, and sections were suspended in 1 ml of prehybridization buffer (50% formamide, 10% dextran sulfate, 2 × SSC, 1 × Denhardt's solution, 50 mM DTT, and 0.5 mg/ml denatured herring sperm DNA), incubated for 2 h at 48 °C. 5-HTT cDNA (a 0.8 kb EcoR1 restriction fragment) [23] or CART cDNA (a 606 bp restriction fragment cloned in our laboratory by PCR amplification using the designed primers, 5′-AGCAGCGAGGAGGTC-CAGAA-3′ and 5′-ACCAACACCATTCGAGGCAT-3′, according to the published mouse CART gene sequence: Accession No. AF148071) probe labeled with $^{35}$S-α-dATP (NEN, PerkinElma, MA, USA) using a random priming kit (Roche Molecular Biochemicals, Indianapolis, IN, USA) was then added into the vials ($1 \times 10^7$ cpm/vial), and hybridized overnight at 48 °C. Following hybridization, the sections were washed at 15 min intervals in decreasing concentrations of SSC (2 ×, 2 ×, 1 ×, 0.5 ×, 0.25 ×, 0.125 ×, 0.125 ×) at 48 °C. The tissue sections were then mounted on gelatin-subbed slides, air-dried, and apposed to Kodak BioMax film (Eastman Kodak, NY, USA) at 4 °C. Exposure times varied from 12 to 48 h to obtain autoradiographic images within a linear range of optical density after development in Kodak D-19 developer. In situ hybridization was carried out on the representative members of each experimental group at the same time under identical conditions, allowing direct comparison of mRNA expression.

### 2.6. Quantitative and statistical analysis

Images on the autoradiographic films were digitized with a Zeiss Stemi-2000 stereoscope attached to a Dage-MTI CCD 72 camera and MCID image analysis system (MCID, Imaging Research, Ontario, Canada). Messenger RNA expression level was determined by quantifying the mean relative optical density of pixels with densities of at least 2 S.D. above the mean density of the image background (mRNA pixels). For each section, the mean background value was subtracted from the mean mRNA pixel value. The mRNA pixel values were averaged across eight sections for CART in situ signals, six for 5-HTT, from each individual rat and the average mRNA value of each rat were then averaged across all rats within each experimental group. The average mRNA values of each experimental group were then converted to relative values to the vehicle-treated control group. All the data was analyzed by one way analysis of variance (ANOVA) and preplanned comparisons with the control were performed by post-hoc Fisher's PLSD test or unpaired *t*-test using StatView software (Abacus, Berkeley, CA).

## 3. Results

### 3.1. 5-HTP effects on food intake and body weight gain

When 5-HTP (100 mg/kg/10 ml) was intraperitoneally administrated 1 h before the lights off, a reduction in spontaneous food intake was detected within 2 h after the injection (Fig. 1A). This reduction effect of an acute 5-HTP administration on food intake was still found at 8 h after the injection. The cumulative food intake remained at a markedly low level during three daily administration of 5-HTP, compared to the saline injection (Fig. 1B). Body weight gain

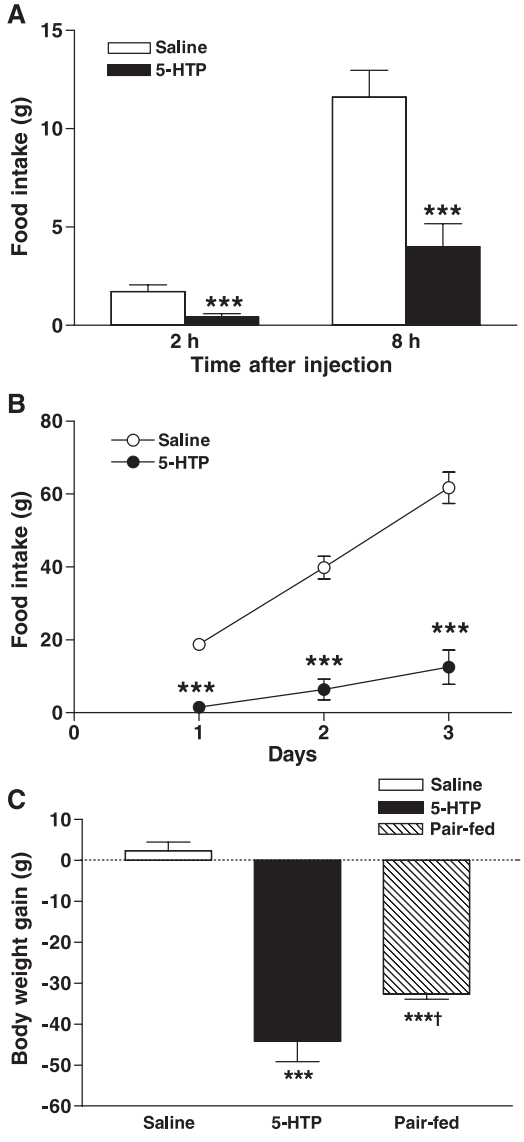


Fig. 1. Food intake and body weight gain during 5-HTP treatment. (A) Food intake after a single 5-HTP injection. A single injection of 5-HTP (100 mg/kg/10 ml) was intraperitoneally given 1 h before the lights off. A significant hypophagic effect of 5-HTP on spontaneous feeding was detected within 2 h and lasted until 8 h after the injection. ***$p < 0.001$ vs. the saline control. (B) Cumulative food intake during three daily 5-HTP (100 mg/kg/day, i.p.). The amount of food intake was consistently reduced (~ 80%) during the injection period. ***$p < 0.001$ vs. the saline control. (C) Body weight gain during three daily 5-HTP. A significant weight loss was detected in both the 5-HTP and the pair-fed group and an additive effect in the body weight loss occurred by 5-HTP administration. ***$p < 0.001$ vs. saline control; $^{\dagger}p < 0.05$ vs. pair-fed.

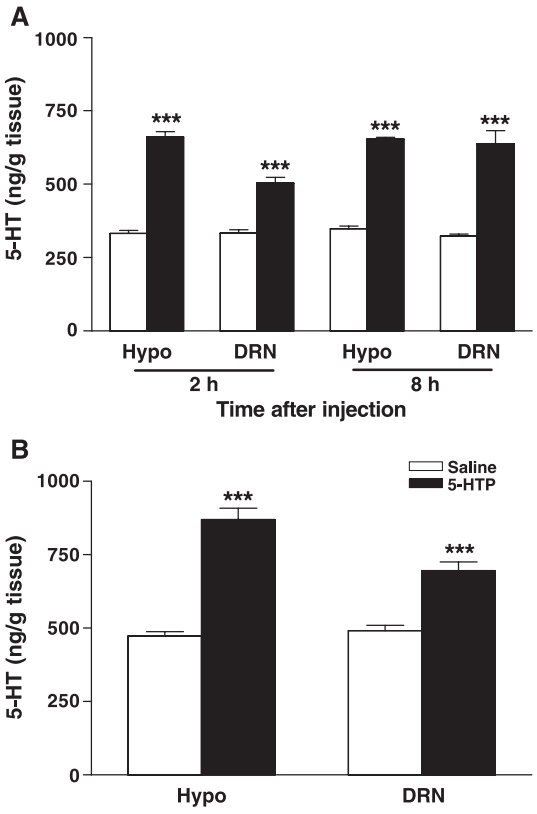


Fig. 2. 5-HT contents of the tissues from the hypothalamus or the raphe nucleus. A. 5-HT levels after a single 5-HTP (100 mg/kg, i.p.). The 5-HT level increased up to 99% in the hypothalamus, 50% in the raphe by 2 h, and 87%, 97% by 8 h after a single 5-HTP injection, respectively. B. 5-HT levels after three daily 5-HTP administrations (100 mg/kg/day, i.p.). 84% of increase in the 5-HT level in the hypothalamus (Hypo), 41% in the raphe nucleus (DRN), was detected by three daily 5-HTP. ***$p<0.001$ vs. saline control.

of the 5-HTP treated group during three daily injections was even lower than the pair-fed group (Fig. 1C), revealing an additive effect produced by the 5-HTP administration on weight loss besides the starvation effect, perhaps due to the increased brain 5-HT level.

### 3.2. Brain 5-HT level

All the 5-HTP treated groups showed significant increases in the 5-HT content not only in the dorsal raphe nucleus where the 5-HT neurons are located, but also in the hypothalamus where the 5-HT output exerts its anorectic effect on feeding, compared to the saline groups at each corresponding time point ($p<0.001$) (Fig. 2). Interestingly, the amount of 5-HT increase in the dorsal raphe tissues became higher at 8 h (~ 97% increase) than at 2 h (~ 50% increase) after the single injection of 5-HTP, and lowered back with the repeated injection (~ 41% increase). This pattern of change in the 5-HT contents was not detected in the hypothalamic tissues (Fig. 2). The mRNA expression of 5-HTT, the 5-HT reuptake transporter, increased in the dorsal raphe nucleus by three daily 5-HTP administration, but not by a single injection, with a statistical significance ($p<0.05$) (Fig. 3). These results together reveal that the 5-HTP administration may induce not only the release but also the synthesis of 5-HT in the brain within a short time, and suggest that an increase in 5-HTT mRNA expression, a negative regulation on 5-HT synthesis as well, may occur as a feed back response to reduce chronically increased level of 5-HT in brain by the repeated 5-HTP.

### 3.3. CART expression in the hypothalamus

The CART mRNA level in the hypothalamic arcuate nucleus was determined by in situ hybridization 2 or 8 h after a single intraperitoneal 5-HTP and 24 h after the last injection of three daily 5-HTP (100 mg/kg/10 ml) (Fig. 4). CART mRNA expression in the arcuate nucleus appeared to decrease by 8 h after the single 5-HTP, but not by pair-fed, although a statistical significance was not found (Fig. 4B). However, the arcuate CART mRNA level after three daily 5-HTP, but not after the 3 days of

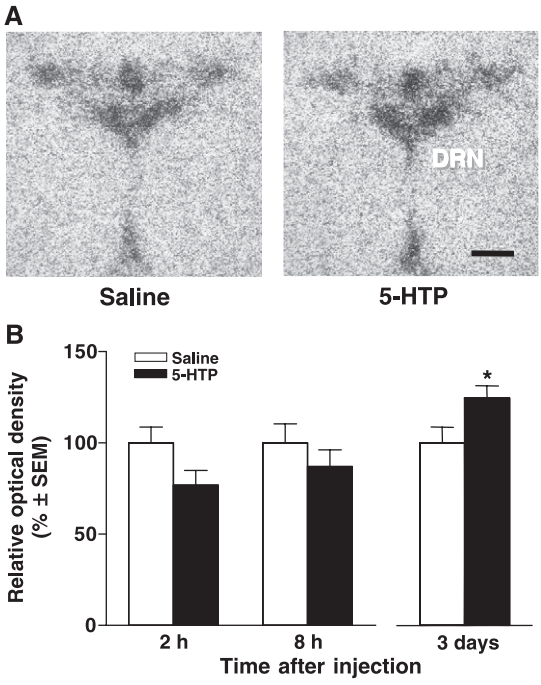


Fig. 3. mRNA levels of 5-HTT, the 5-HT reuptake transporter, in the dorsal raphe nucleus (DRN). (A) Autoradiography of 5-HTT in situ hybridization. 5-HTT in situ signals in the dorsal raphe nucleus appeared to be stronger in the three daily 5HTP treated group, compared to the saline control. (B) Relative optical density of the in situ signals on autoradiographic films. 5-HTT mRNA level appeared to decrease by a single 5-HTP, but a statistical significance was not found either at 2 h ($p=0.076$) or 8 h ($p=0.696$) after the injection. However, the relative optical density of 5-HTT in situ signal increased up to 24% in the three daily 5-HTP group with a statistical significance. *$p<0.05$ vs. saline control. Scale bar: 300 μm.

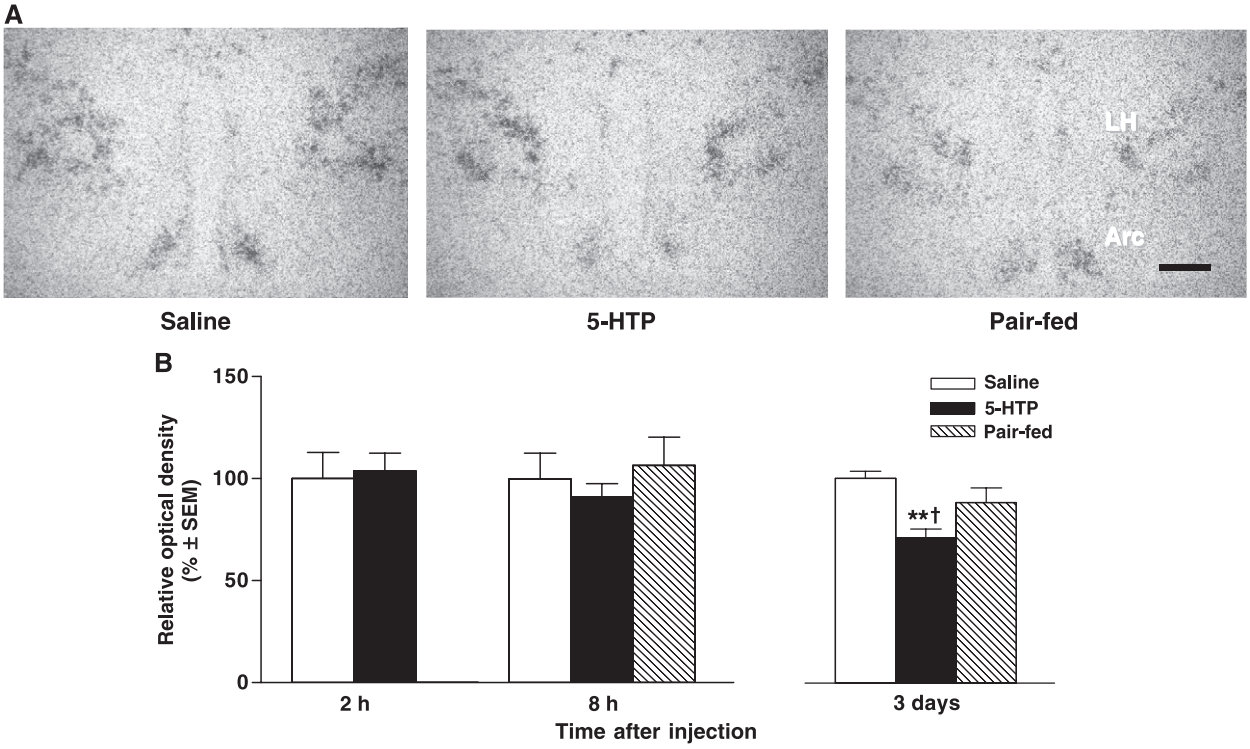


Fig. 4. CART mRNA levels in the hypothalamus. (A) Autoradiography of CART in situ hybridization after three daily 5-HTP (100 mg/kg) or pair-fed. (B) Relative optical density of the CART in situ signals on autoradiographic films. A significant decrease in CART mRNA level was detected in the arcuate nucleus by three daily 5-HTP, but not by a single, compared to each saline or pair-fed group. $^{**}p<0.01$ vs. saline control; $^{\dagger}p<0.05$ vs. pair-fed. Scale bar: 1 mm.

pair-fed, was significantly ($p=0.0012$) decreased, compared to the saline free fed group (Fig. 4A,B). These results reveal that the reduction in food intake, a satiate effect of 5-HTP administration, may not be sufficient to make a significant decrease in the arcuate CART expression level.

### 3.4. Plasma leptin level

The plasma level of leptin, a signal linking the adipose tissue status with the central circuits of feeding control, was examined to determine if it correlates with the reduction in food intake and body weight induced by 5-HTP. The plasma leptin level was not significantly decreased by 8 h of 5-HTP administration, however, its pair-fed group showed a marked reduction (~ 46% decrease) (Fig. 5A). After three daily 5-HTP, both the 5-HTP and the pair-fed group showed a remarkable decrease in the plasma leptin level, compared to the free fed saline group (Fig. 5B), however, the 5-HTP group still showed a higher leptin level than the pair-fed group with a statistical significance ($p<0.05$) from unpaired $t$-test. This was despite the fact that the 5-HTP group lost their body weight even more than the pair-fed group during the experimental period (Fig. 1). These results reveal that the plasma leptin level may negatively correlate with body weight gain in the 5-HTP group.

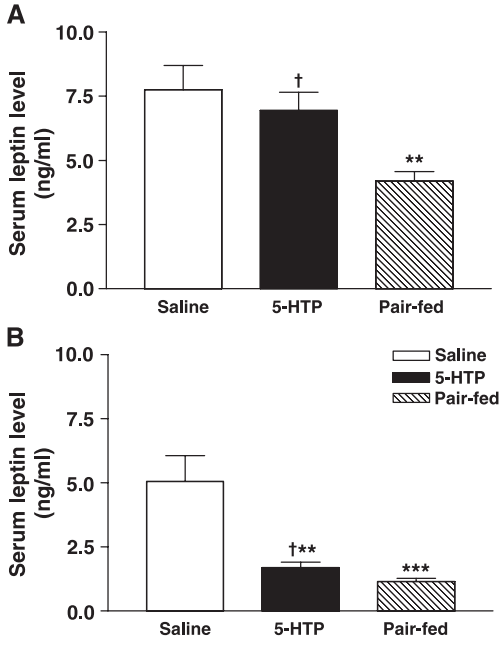


Fig. 5. Plasma leptin level 8 h after a single injection (A) or 24 h after three daily injections of 5-HTP (100 mg/kg, i.p.) (B). The plasma leptin level significantly decreased by 8 h of pair-fed, but not by a single 5-HTP (A). Both three daily 5-HTP and its pair-fed markedly decreased the plasma leptin, compared to the saline free fed group. However, the plasma leptin level of the 5-HTP group was still higher than of the pair-fed group with a statistical significance from unpaired $t$-test (B). $^{**}p<0.01$ vs. saline control; $^{***}p<0.001$ vs. saline control; $^{\dagger}p<0.05$ vs. pair-fed.

## 4. Discussion

### 4.1. Effects of systemic 5-HTP on the brain 5-HT system

We demonstrated that 5-HT levels in both the hypothalamus and the dorsal raphe nucleus increased by 5-HTP treatment, which was consistent with the result previously reported by others that the administration of 5-HTP causes an immediate increase of 5-HT in the hypothalamic dialysates and this effect is long lasting and dose-dependent [20]. The present results also showed that mRNA expression of 5-HTT, the 5-HT reuptake transporter, in the dorsal raphe nucleus was increased by chronic, but not by an acute, 5-HTP administration, and this is the first finding regarding the effect of 5-HTP administration on 5-HTT gene expression. Increased expression of 5-HTT in the dorsal raphe nucleus could be a negative feed back response to reduce the chronically increased synaptic 5-HT level by the repeated 5-HTP. It was reported that the 5-HTT mRNA expression in the raphe nucleus decreases with the depletion of brain 5-HT by chronic para-chlorophenylalanine treatment [28,29]. An interesting point of this study is that the increased amount of 5-HT content varied in the raphe, but remained at a consistent level in the hypothalamus, by 5-HTP administration. That is, 5-HT content in the raphe nucleus, where most of brain 5-HT neurons are located, increased by an acute 5-HTP in a time dependent manner and the amount of increase became reduced after the repeated 5-HTP, however, this pattern of changes was not detected in the hypothalamus. Taken all together, these results suggest that the brain 5-HT system is activated by 5-HTP administration, and that a negative interaction on 5-HT synthesis as well as a positive on 5-HTT may be produced by the repeated 5-HTP in the raphe nucleus as a feed back response to an increased 5-HT level in the hypothalamus.

5-HTT mRNA level in the dorsal raphe nucleus in the pair-fed group given 3 days of food restriction did not differ from the free fed saline group, in our preliminary experiment (data not shown). This finding concurs with our previous report that 24 h food deprivation did not alter the 5-HTT mRNA level in the dorsal raphe of mice [30]. Furthermore, long-term food restriction has been reported to decrease the activity of the brain 5-HT system, such as a decrease in 5-HTT level [31] as well as in 5-HTT density [32]. Therefore, it is unlikely that the pair-fed, food restricted, groups in the present study might have an increased activity in the brain 5-HT system, which would have influenced on the results observed. Also, it can be concluded that the increase in 5-HTT mRNA expression in the 5-HTP group could not be a consequence of the reduced food intake and the body weight loss, but rather of an interaction with increased brain 5-HT level.

### 4.2. Effects of systemic 5-HTP on food intake and weight gain

It was shown in this study that 5-HTP consistently produced a significant reduction in both the food consumption and weight gain during the experimental period, compared to the saline injections. This may occur as a result of the increased activity of brain 5-HT system detected with the 5-HTP administrations, in accordance with previous reports on the anorectic action of the brain 5-HT [9]. Interestingly, the pair-fed group, which received the same amount of food that the 5-HTP group had consumed, showed a smaller loss in body weight than the 5-HTP group. This suggests that 5-HTP may decrease body weight through its inhibitory effect on energy intake and also an additional effect on body weight loss, perhaps an increase in energy expenditure, may occur by the systemic 5-HTP. This is supported by previous reports showing that 5-HT may increase the resting energy expenditure [10,33].

### 4.3. Plasma leptin level

Differential effect on the plasma leptin level was detected in the 5-HTP and pair-fed group in this study. The plasma leptin level was remarkably decreased by 3 days of food restriction, pair-fed, as was expected from the significant body weight loss. The plasma leptin level of the 5-HTP group was also decreased, compared to the saline group, however, still higher than the pair-fed group, although the body weight loss was even larger. No report has been made regarding a stimulatory effect of the brain 5-HT on leptin synthesis or release in the adipose tissues. Rather it seems that the central 5-HT participates in the control of feeding with a separate system from the leptin action [9]. On the contrary, there was a report suggesting a role of peripheral 5-HT in the plasma leptin level, which showed that the systemic, but not the central, administration of 5-HTP acutely increased the plasma leptin level [19]. Yamada et al. [19] reported that the plasma leptin level acutely increased from 30 min to 3 h after the systemic 5-HTP. This report concurs our result that the plasma leptin level was not decreased by 8 h after a single 5-HTP, while the pair-fed group showed a marked reduction during the same time period. Therefore, it can be suggested that the systemic 5-HTP in this study may produce an additional effect on body weight loss, at least partly, through its stimulatory action on the plasma leptin level.

### 4.4. Hypothalamic CART expression

In the present study, 5-HTP administration produced a time dependent decrease in CART mRNA expression in the arcuate nucleus. The decrease in CART mRNA expression level appeared to start by 8 h after a single 5-HTP, and

became significantly clear by three daily 5-HTP. This effect on CART expression may have occurred by the hypophagic effect of 5-HTP administration [8,33], which was revealed as ~ 80% reduction in food intake accompanied by a significant weight loss, compared to the saline control. It was reported that CART mRNA expression in the hypothalamus is down-regulated by fasting and up-regulated by leptin administration [4]. However, the pair-fed groups of each 5-HTP treatment, which received the same amount of food (~ 20% of the free fed control) consumed by the 5-HTP group, did not show any significant decrease in the arcuate expression of CART, compared to the saline free fed control. These results indicate that ~ 80% of food restriction alone may not be sufficient to produce a significant decrease in the arcuate expression of CART mRNA, and that the increased activity of the brain 5-HT system by 5-HTP treatment may possibly produce an additive effect on the down-regulation of the arcuate CART expression. This is the first report showing that the brain 5-HT system may negatively interact with the CART gene expression in the hypothalamic arcuate nucleus.

It has been reported that anorectic (*anx*/*anx*) mouse showing the typical behaviors of serotonin syndrome [34] contains 5-HT hyperinnervation in the whole brain [17] and shows an increased activity of the brain 5-HT system [30]. This mouse was also reported to have a decreased expression level of the hypothalamic CART as well as pro-opiomelanocortin and NPY [15,18,35]. Johansen et al. [18] suggested that the decreased expression of the hypothalamic CART mRNA in the anorectic (*anx*/*anx*) mouse may be due to the decreased level of plasma leptin induced by serious weight loss. A possible implication of the plasma leptin level in the down-regulation of arcuate CART mRNA expression should be considered in the present results as well. Furthermore, it was reported that CART mRNA expression in the hypothalamus was decreased in obese (*ob*/*ob*) mouse lacking functional leptin in their plasma as well as in Zucker (*fa*/*fa*) rat lacking the functional receptor for leptin [4]. It seems that the decreased expression of the hypothalamic CART mRNA may correlate with the decreased activity of leptin signaling. However, this contrasts with the present results demonstrating that the pair-fed groups of the 8 h or three daily 5-HTP group, which showed even bigger reductions in the plasma leptin level than each corresponding 5-HTP group, did not show any significant decreases in the hypothalamic CART expression, on the contrary to the significant decrease produced in the 5-HTP groups. Therefore, it can be concluded that there may be an another pathway other than the leptin signaling which regulates the hypothalamic CART mRNA level, and the brain 5-HT, as a part of this non-leptin mediated pathway, appears to exert an inhibitory role in the hypothalamic CART expression.

In summary, the brain 5-HT contents and 5-HTT gene expression increased by systemic administration of 5-HTP (100 mg/kg) with a positive correlation, suggesting an increased activity of the brain 5-HT system. The systemic 5-HTP produced an additional effect on body weight loss, likely by its peripheral effect on the plasma leptin level. CART mRNA expression in the hypothalamic arcuate nucleus was down-regulated by the systemic 5-HTP administration, in which the plasma leptin level did not show a positive correlation with the CART expression level. These results indicate that the increased activity of the brain 5-HT system may negatively interact with the hypothalamic CART mRNA expression, as a part of non-leptin mediate regulatory pathway in the control of CART gene expression in the hypothalamus.

## Acknowledgements

The authors thank Mr. Gun Tae Kim for his excellent technical help with HPLC analysis. This work was supported by the Neurobiology Research Program from the Korea Ministry of Science and Technology (#M1-01-08-00-0021).

## References

[1] Douglass J, Mckinzie AA, Couceyro P. PCR differential display identifies a rat brain mRNA that is transcriptionally regulation by cocaine and amphetamine. J Neurosci 1995;15:2471–81.
[2] Couceyro PR, Koylu EO, Kuhar MJ. Further studies on the anatomical distribution of CART by in situ hybridization. J Chem Neuroanat 1997;12:229–41.
[3] Vrang N, Larsen PJ, Clausen JT, Kristensen P. Neurochemical characterization of hypothalamic cocaine-amphetamine-regulated transcript neurons. J Neurosci 1999;19(RC5):1–8.
[4] Kristensen P, Judge ME, Thim L, Rlbel U, Christjansen KN, Wulff BS, et al. Hypothalamic CART is a new anoretic peptide regulated by leptin. Nature 1998;393:72–6.
[5] Lambert PD, Couceyro PR, McGirr KM, Dall-Vechia SE, Smith Y, Kuhar MJ. CART peptides in the central control of feeding and interactions with neuropeptide Y. Synapse 1998;29:293–8.
[6] Aja S, Sahandy S, Ladenheim EE, Schwartz GJ, Moran TH. Intracerebroventricular CART peptide reduces food intake and alters motor behavior at a hindbrain site. Am J Physiol, Regul Integr Comp Physiol 2001;281:R1862–7.
[7] Orlando G, Brunetti L, Di Nisio C, Michelotto B, Recinella L, Ciabattoni G, et al. Effects of cocaine- and amphetamine-regulated transcript peptide, leptin and orexins on hypothalamic serotonin release. Eur J Pharmacol 2001;430:269–72.
[8] Curzon G. Serotonin and appetite. Ann N Y Acad Sci 1990;600:521–30.
[9] Halford JC, Blundell JE. Separate systems for serotonin and leptin in appetite control. Ann Med 2000;32:222–32.
[10] Leibowitz SF, Alexander JT. Hypothalamic serotonin in control of eating behavior, meal size, and body weight. Biol Psychiatry 1998;44:851–64.
[11] Voigt JP, Schade R, Fink H, Hörtnagl H. Role of 5-HT1A receptors in the control of food intake in obese Zucker rats of different ages. Pharmacol Biochem Behav 2002;72:403–9.
[12] Collin M, Backberg M, Önnestam K, Meister B. 5-HT1A receptor immunoreactivity in hypothalamic neurons involved in body weight control. NeuroReport 2002;13:945–51.
[13] Dryden S, Wang Q, Frankish HM, Pickavance L, Williams G. The

serotonin (5-HT) antagonist methysergide increases neuropeptide Y (NPY) synthesis and secretion in the hypothalamus of the rat. Brain Res 1995;699:12–8.
[14] Dryden S, Frankish HM, Wang Q, Williams G. Increased feeding and neuropeptide Y (NPY) but not NPY mRNA level in the hypothalamus of the rat following central administration of the serotonin synthesis inhibitor *p*-chlorophenylalanine. Brain Res 1996;724:232–7.
[15] Jahng JW, Houpt TA, Kim SJ, Joh TH, Son JH. Neuropeptide Y mRNA and serotonin innervation in the arcuate nucleus of anorexia mutant mice. Brain Res 1998;790:67–73.
[16] Currie PJ, Coiro CD, Niyomchai T, Lira A, Farahmand F. Hypothalamic paraventricular 5-hydroxytryptamine: Receptor-specific inhibition of NPY-stimulated eating and energy metabolism. Pharmacol Biochem Behav 2002;71:709–16.
[17] Son JH, Baker H, Park DH, Joh TH. Drastic and selective hyperinnervation of central serotonergic neurons in a lethal neurodevelopmental mouse mutant, Anorexia (*anx*). Brain Res Mol Brain Res 1994;25:129–34.
[18] Johansen JE, Broberger C, Lavebratt C, Johansson C, Kuhar MJ, Hokfelt T, et al. Hypothalamic CART and serum leptin levels are reduced in the anorectic (*anx*/*anx*) mouse. Brain Res Mol Brain Res 2000;84:97–105.
[19] Yamada J, Ujikawa M, Sugimoto Y. Serum leptin levels after central and systemic injection of a serotonin precursor, 5-hydroxytryptophan, in mice. Eur J Pharmacol 2000;406:159–62.
[20] Gartside SE, Cowen PJ, Sharp T. Effect of 5-hydroxy-L-tryptophan on the release of 5-HT in rat hypothalamus in vivo as measured by microdialysis. Neuropharmacology 1992;31:9–14.
[21] Ju CY, Tsai CT. Serotonergic mechanisms involved in the suppression of feeding by 5-HTP in rats. Chin J Physiol 1995;38:235–40.
[22] Mokler DJ, Sullivan SA, Winterson BJ. Behaviors induced by 5-hydroxytryptophan in neonatal, preweaning, postweaning, and adult Sprague–Dawley rats. Pharmacol Biochem Behav July 1992;42:413–9.
[23] Nisijima K, Yoshino T, Yui K, Katoh S. Potent serotonin (5-HT)(2A) receptor antagonists completely prevent the development of hyperthermia in an animal model of the 5-HT syndrome. Brain Res 2001 Jan 26;890:23–31.
[24] Nisijima K, Shioda K, Yoshino T, Takano K, Kato S. Diazepam and chlormethiazole attenuate the development of hyperthermia in an animal model of the serotonin syndrome. Neurochem Int 2003 (July);43:155–64.
[25] Indra B, Tadano T, Nakagawasai O, Arai Y, Yasuhara H, Ohizumi Y, et al. Suppressive effect of nantenine, isolated from Nandina domestica Thunberg, on the 5-hydroxy-L-tryptophan plus clorgyline-induced head-twitch response in mice. Life Sci 2002 April 19;70:2647–56.
[26] Wagner J, Vitali P, Palfreyman MG, Zraika M, Huot S. Simultaneous determination of 3,4-dihydroxyphenylalanine, 5-hydroxytryptophan, dopamine, 4-hydroxy-3-methoxyphenylalanine, norepinephrine, 3,4-dihydroxyphenylacetic acid, homovanillic acid, serotonin, and 5-hydroxyindoleacetic acid in rat cerebrospinal fluid and brain by high-performance liquid chromatography with electrochemical detection. J Neurochem 1982;38:1241–54.
[27] Paxinos G, Watson C. The rat brain in stereotaxic coordinate. Academic Press; 1986.
[28] Linnet K, Koed K, Wiborg O, Gregersen N. Serotonin depletion decreases serotonin transporter mRNA levels in rat brain. Brain Res 1995;697:251–3.
[29] Yu A, Yang J, Pawlyk AC, Tejani-Butt SM. Acute depletion of serotonin down-regulates serotonin transporter mRNA in raphe neurons. Brain Res 1995;688:209–12.
[30] Jahng JW, Houpt TA, Joh TH, Son JH. Differential expression of monoamine oxidase A, serotonin transporter, tyrosine hydroxylase and norepinephrine transporter mRNA by anorexia mutation and food deprivation. Brain Res Dev Brain Res 1998;107:241–6.
[31] Haider S, Haleem DJ. Decreases of brain serotonin following a food restriction schedule of 4 weeks in male and female rats. Med Sci Monit 2000;6:1061–7.
[32] Huether G. Acute regulation and long-term modulation of presynaptic serotonin output. Adv Exp Med Biol 1999;467:1–10.
[33] Walsh KM, Leen E, Lean ME. The effect of sibutramine on resting energy expenditure and adrenaline-induced thermogenesis in obese females. Int J Obes Relat Metab Disord 1999;23:1009–15.
[34] Maltais LJ, Lane PW, Beamer WG. Anorexia, a recessive mutation causing starvation in preweanling mice. J Hered 1984;75:468–72.
[35] Broberger C, Johansen J, Brismar H, Johansson C, Schalling M, Hokfelt T. Changes in neuropeptide Y receptors and pro-opiomelanocortin in the anorexia (*anx*/*anx*) mouse hypothalamus. J Neurosci 1999;9:7130–9.