

# Effects of food deprivation on the hypothalamic feeding-regulating peptides gene expressions in serotonin depleted rats

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**Abstract** We examined the effects of serotonin (5-HT) depletion induced by peripheral injection of 5-HT synthesis inhibitor *p*-chlorophenylalanine (PCPA) on the expression of feeding-regulating peptides expressions by using in situ hybridization histochemistry in adult male Wistar rats. PCPA pretreatment had no significant effect on basal levels of *oxytocin*, *corticotropin-releasing hormone (CRH)*, *thyrotropin-releasing hormone (TRH)*, *pro-opiomelanocortin (POMC)*, *cocaine and amphetamine-regulated transcript (CART)*, *neuropeptide-Y (NPY)*, *agouti-related protein (AgRP)*, *melanin-concentrating hormone (MCH)* or *orexin* in the hypothalamus. Food deprivation for 48 h caused a significant decrease in *CRH*, *TRH*, *POMC*, and *CART*, and a significant increase in *NPY*, *AgRP* and *MCH*. After PCPA treatment, *POMC* and *CART* did not decrease despite food deprivation. *NPY* was significantly increased by food

deprivation with PCPA, but was attenuated compared to food deprivation without PCPA. These results suggest that the serotonergic system in the hypothalamus may be involved in the gene expression of *POMC*, *CART*, and *NPY* related to feeding behavior.

**Keywords** Serotonin · Feeding · Neuropeptides · Hypothalamus · *p*-chlorophenylalanine

## Introduction

Many physiological behaviors, including emotion, memory, learning, awakening, attention, thermoregulation, and feeding, are manipulated by the serotonergic system [1–4]. With regard to feeding behavior, although its regulation by serotonin (5-HT) is broadly well described, the mechanisms or central pathways that mediate this behavior are still poorly understood. While current approaches to identifying the mechanisms of feeding regulation have focused on feeding-regulating neuropeptides [5], possible interactions between monoamine and neuropeptides in appetite regulation have been clarified [6, 7].

*p*-chlorophenylalanine (PCPA) depletes brain serotonin (5-HT) by synthetic inhibition [8, 9], of which systemic pretreatment for 2 days resulted in 95 % depletion in hypothalamic serotonin [10]. This pharmaceutical enables us to explicate the interactions between 5-HT and feeding-regulating neuropeptides. Generally, anorexigenic peptides, such as corticotropin-releasing hormone (CRH) [11] and thyrotropin-releasing hormone (TRH) [12, 13], are down-regulated by food deprivation; conversely, orexigenic peptides, such as neuropeptide-Y (NPY) [14] and melanin-concentrating hormone (MCH) [15], are up-regulated. If the feeding-regulating neuropeptides do not

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change despite food deprivation after PCPA pretreatment, it can be referred that the neuropeptides may have possible interactions with 5-HT.

Several studies have demonstrated the correlations between 5-HT and hypothalamic feeding-regulating neuropeptides [8, 9, 16–18], although which kind of feeding-regulating peptides are affected by the serotonergic system is unclear. Here, we comprehensively examined the interactions between the serotonergic system and feeding-regulating neuropeptides in the hypothalamus after 48 h food deprivation with or without PCPA pretreatment, using *in situ* hybridization histochemistry (IHC) in rats.

## Materials and methods

### Animals

Adult male Wistar rats (180–190 g body weight) were individually housed and maintained in temperature controlled (23–25 °C) conditions under a 12.12 h light/dark cycle (lights on at 0700 hours). All experiments were performed in strict accordance with the guidelines on the use and care of laboratory animals issued by the Physiological Society of Japan, and were approved by the Ethics Committee of Animal Care and Experimentation of University of Occupational and Environmental Health.

### Test substance

PCPA (Sigma-Aldrich Japan, Tokyo, Japan) was dissolved in 0.9 % sterile physiological saline (Otsuka Pharmaceutical, Tokyo, Japan) (0.6 mg/1 mL).

### Determination of hypothalamic monoamine concentrations

Hypothalamic concentrations of noradrenaline (NA), dopamine (DA), and 5-HT were measured by high-performance liquid chromatography with electrochemical detection (HPLC-ECD). PCPA or saline were administered by intraperitoneally (i.p.) injection daily for 2 days. The rats were decapitated 48 h after second saline ( $n = 8$ ) or PCPA ( $n = 8$ ) treatment followed by removal of the hypothalamus. Briefly, samples were frozen onto dry-ice and stored at  $-80$  °C before the measurements. Samples were homogenized in 0.2 M perchloric acid and centrifuged (8,000g) at 4 °C for 30 min. The supernatant was collected and analyzed with HPLC-ECD system (Hitachi, Japan). HPLC-ECD conditions were a modified method of Wetherell et al. [19]. Briefly, separations were performed using a  $4.6 \times 150$  mm ODS  $C_{18}$  column. The mobile

phase consisted of 0.1 M  $\text{Na}_2\text{PO}_4$ , 0.8 mM OSA, 0.5 M EDTA, and 10 % methanol, and was adjusted to pH 3.63 with phosphoric acid. Column temperature was 40 °C, flow rate 1.0 mL/min, and the detector was set at a potential of +0.75 V relative to an Ag/AgCl reference electrode. The working standard solution was prepared in 0.2 M perchloric acid containing 0.5 mM EDTA and 0.05 mg/mL DHBA was stored at  $-80$  °C.

### Experimental procedure

All the rats had *ad libitum* access to water throughout the experiments. The rats were divided into four groups: saline + *ad libitum* access to food (SAF,  $n = 13$ ), saline + food deprivation for 48 h (SFD,  $n = 13$ ), PCPA + *ad libitum* access to food (PAF,  $n = 14$ ), and PCPA + food deprivation for 48 h (PFD,  $n = 14$ ). Saline (10 mL/kg body weight as a single daily dose) or PCPA (200 mg/10 mL/kg body weight as a single daily dose) was administered i.p. at day 0 and day 1 (0900–1000 hours). After the administration of saline or PCPA at day 1, food was deprived in SFD and PFD. Body weights and food intake in all the experimental groups were measured every 24 h from day 1 to day 6.

On day 3, some of those rats (SAF,  $n = 7$ , SFD,  $n = 7$ , PAF,  $n = 8$ , PFD,  $n = 8$ ) were decapitated immediately after the treatment without being anesthetized, followed by prompt removal of the brain onto dry ice, then storing at  $-80$  °C. Trunk blood samples were taken during decapitation, and were collected into chilled reaction tubes (Greiner Bio-One) containing an aprotinin/EDTA mixture. The blood samples were immediately centrifuged for 10 min at 4 °C, 1,000g, after which, a 15- $\mu\text{L}$  sample of plasma was taken for measuring plasma osmolality (P-Osm) using a ONE-TEN osmometer (FISKE, Norwood, MA, USA), 500  $\mu\text{L}$  for plasma 5-HT (SRL, Tokyo, Japan), 500  $\mu\text{L}$  for plasma leptin (SRL), 1,000  $\mu\text{L}$  for plasma active and desacyl ghrelin (SRL), and 10  $\mu\text{L}$  for measuring plasma glucose (PG) using a Medisafe Reader GR-101 (Terumo, Tokyo, Japan). Finally, 100  $\mu\text{L}$  of 1 M HCl was added to each tube for measuring active and desacyl ghrelin in order to protect against decomposition.

### In situ hybridization histochemistry

The removed brains were cut into 12- $\mu\text{m}$  slices and thaw-mounted on gelatin/chrome alum-coated slides. The locations of the hypothalamic areas, including the supraoptic nucleus (SON), paraventricular nucleus (PVN), arcuate nucleus (ARC), and lateral hypothalamic area (LHA), were determined according to coordinates of the rat brain atlas.

<sup>35</sup>S 3'- end-labeled deoxyoligonucleotide complementary to transcripts encoding *oxytocin*, *CRH*, *TRH*, *pro-opiomelanocortin (POMC)*, *cocaine and amphetamine-regulated transcript (CART)*, *NPY*, *agouti-related protein (AgRP)*, *MCH*, and *orexin* were used (*oxytocin* probe sequence, 5'-CTC GGA GAA GGC AGA CTC AGG GTC GCA GGC-3'; *CRH* probe sequence, 5'-CAG TTT CCT GTT GCT GTG AGC TTG CTG AGC TAA CTG CTC TGC CCT GGC-3'; *TRH* probe sequence, 5'-GTC TTT TTC CTC CTC CTC CCT TTT GCC TGG ATG CTG CGC TTT TGT GAT-3'; *POMC* probe sequence, 5'-TGG CTG CTC TCC AGG CAC CAG CTC CAC ACA TCT ATG GAG G-3'; *CART* probe sequence, 5'-TCC TTC TCG TGG GAC GCA TCA TCC ACG GCA GAG TAG ATG TCC AGG-3'; *NPY* probe sequence, 5'-CAA ATG GAT GAT TGG TCA TTT CAA CAT AGA GTT GGG GGC TTG CT-3'; *AgRP* probe sequence, 5'-CGA CGC GGA GAA CGA GAC TCG CGG TTC TGT GGA TCT AGC ACC TCT GCC-3'; *MCH* probe sequence, 5'-CCA ACA GGG TCG GTA GAC TCG TCC CAG CAT-3'; and *orexin* probe sequence, 5'-TCC TCA TAG TCT GGA GGC AGG TGG AAG GGT TCC CCA CTG CTA GTG-3') The specificity of these probes were confirmed by previous studies [20, 21].

The probe was 3'-end-labeled using terminal deoxynucleotidyl transferase and [<sup>35</sup>S] dATP. The in situ hybridization protocol has been previously described in detail [22]. Briefly, sections were fixed in 4 % (w/v) formaldehyde for 5 min and incubated in saline containing 0.25 % (v/v) acetic anhydride and 0.1 M triethanolamine for 10 min and then dehydrated, delipidated in chloroform, and partially rehydrated. Hybridization was carried out overnight at 37 °C in 45 µL of hybridization buffer under a Nescofilm (Bando Kagaku, Osaka, Japan) cover slip. A total count of  $1 \times 10^5$  c.p.m. for the *oxytocin* transcripts and  $1 \times 10^6$  c.p.m. for the *CRH*, *TRH*, *POMC*, *CART*, *NPY*, *AgRP*, *MCH* and *orexin* transcripts were used per slide. After hybridization, the sections were washed 4 times with SSC (150 mM NaCl and 15 mM sodium citrate) for 1 h at 55 °C and for an additional hour with two changes of SSC at room temperature. The hybridized sections containing hypothalamus were exposed for autoradiography (Hyperfilm; Amersham, Bucks, UK) for 6 h for the *oxytocin* probe, 5 days for the *MCH* and *orexin* probe, and 1 week for the *CRH*, *TRH*, *POMC*, *CART*, *NPY*, and *AgRP* probe. The resulting images were analyzed by computerized densitometry using a MCID imaging analyzer (Imaging Research, Ontario, Canada). The mean optical densities (OD) of the autoradiographs were measured by comparison with simultaneously exposed <sup>14</sup>C-labeled microscale samples (Amersham) and represented in arbitrary units setting the mean OD obtained from control rats.

## Statistical analysis

The mean  $\pm$  SEM was calculated from the results of the change in body weight, cumulative food intake, plasma measurements, and ISH studies. In the results of ISH, the expression levels of the genes were expressed as a percentage of PAF. All data were analyzed by one-way ANOVA followed by a Bonferroni-type adjustment for multiple comparisons (Origin Pro v.8.5 J; Lightstone, Tokyo, Japan). Statistical significance was set at  $P < 0.05$ .

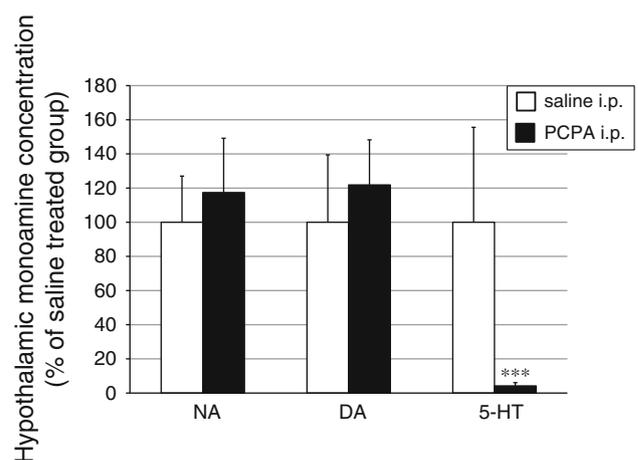
## Results

### Hypothalamic monoamine concentrations after PCPA treatment

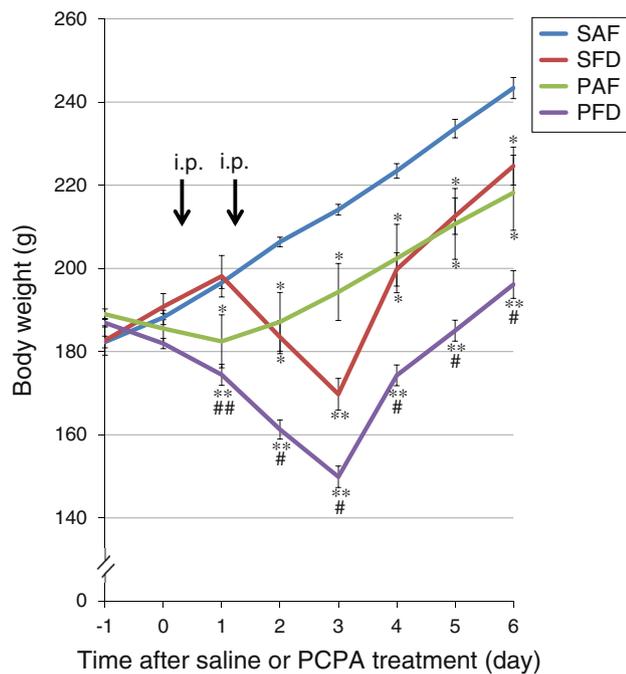
Hypothalamic NA, DA, and 5-HT were measured using HPLC (Fig. 1). No statistically differences were observed in hypothalamic NA and DA; however, hypothalamic 5-HT levels nearly depleted in PCPA-treated group compared to saline-treated group (Fig. 1).

### Changes in body weight

The body weight of each group was measured from day 1 to day 6 (Fig. 2). The body weight gradually increased during the experiments in SAF (Fig. 2). The body weight in PAF gradually increased after day 1, but was statistically different compared to SAF after day 1. The body weight decreased after PCPA administration (day 1) in PFD and PAF compared to SAF (Fig. 2). A decrease in body weight was observed in SFD and PFD after starting food



**Fig. 1** Hypothalamic monoamine concentration after i.p. administration of saline or PCPA. These data were measured by HPLC. The amount of each monoamine level represents % from saline group. Data are presented as mean  $\pm$  SEM. \*\*\* $P < 0.001$  vs. saline group. NA noradrenaline, DA dopamine, 5-HT serotonin



**Fig. 2** Changes in body weight changes in body weight from day 1 to day 6. Arrows indicate saline or PCPA i.p. administration. Data are presented as mean  $\pm$  SEM. \* $P < 0.05$  vs. SAF, \*\* $P < 0.01$  vs. SAF and PAF, # $P < 0.05$  vs. SFD, ### $P < 0.01$  vs. SFD. SAF saline + ad libitum access to food, SFD saline + food deprivation for 48 h, PAF PCPA + ad libitum access to food, PFD PCPA + food deprivation for 48 h

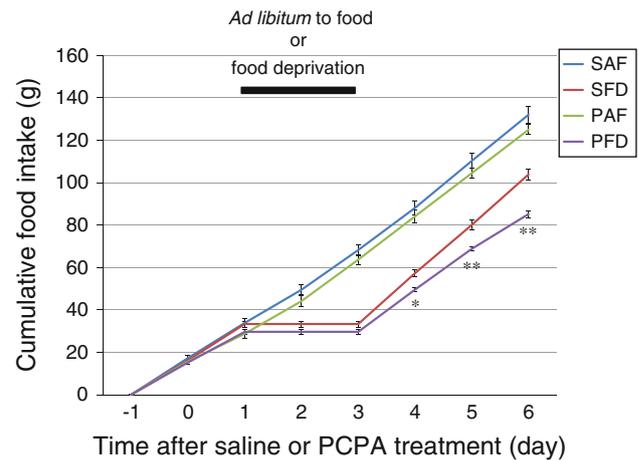
deprivation (Fig. 2). There were no statistical differences between the body weight of SFD and PAF after day 4.

#### Food intake

Cumulative food intake during the experiment was measured from day 1 to day 6 (Fig. 3). Cumulative food intake was comparable in all among the groups at day 0 and day 1. Cumulative food intake in SFD and PFD was null after starting food deprivation (Fig. 3). Cumulative food intake in PFD decreased at days 4, 5, and 6 compared to SFD (Fig. 3).

#### Plasma measurement

PG, P-Osm, 5-HT, leptin, and active/desacyl ghrelin concentration at day 3 were measured. PG in SFD ( $62 \pm 2.4$  mg/dL) and PFD ( $75 \pm 4.1$  mg/dL) decreased significantly compared to that in SAF ( $108 \pm 1.8$  mg/dL) and PAF ( $105 \pm 3.9$  mg/dL). No statistical differences were seen between SAF and PAF or between SFD and PFD. There were no significant differences in P-Osm among all the experimental groups (SAF:  $311 \pm 1.3$  mOsm/kg; SFD:  $310 \pm 2.78$  mOsm/kg; PAF:  $307 \pm 2.2$  mOsm/kg; PFD:  $310 \pm 1.7$  mOsm/kg). Plasma 5-HT level significantly

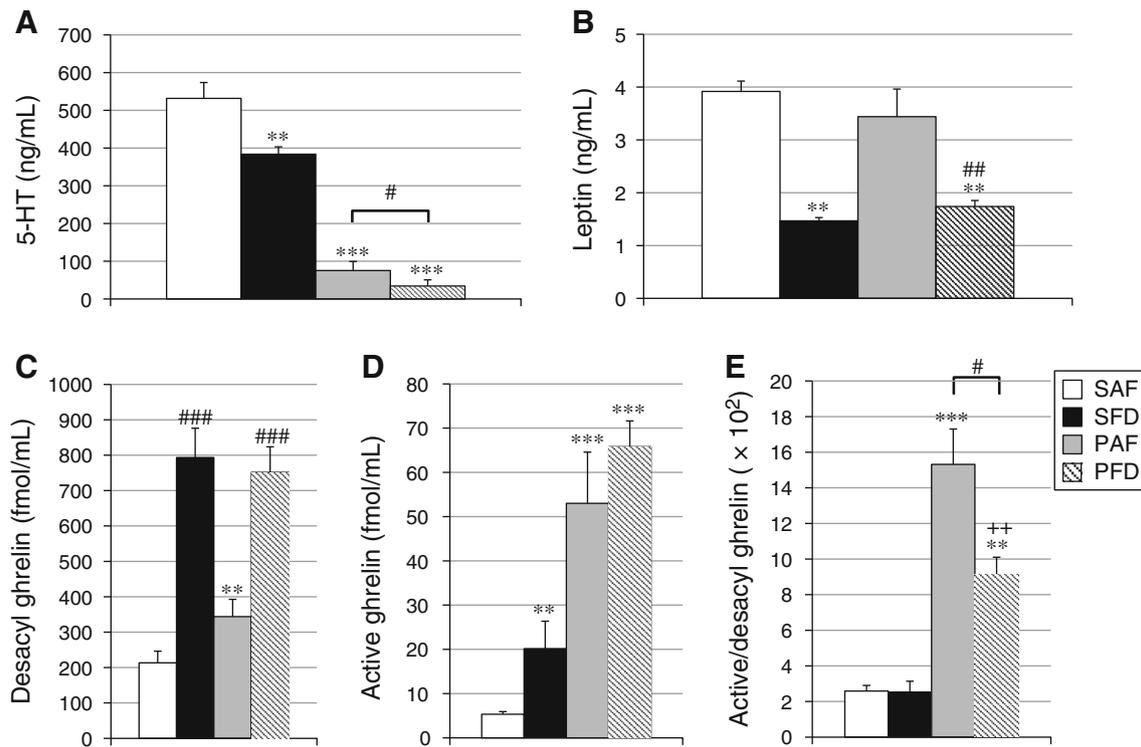


**Fig. 3** Changes in food intake Cumulative food intake (g) from day 1 to day 6. Data are presented as mean  $\pm$  SEM. \* $P < 0.05$  vs. SFD, \*\* $P < 0.01$  vs. PFD. Saline or PCPA was i.p. administered at day 0 and day 1

decreased in SFD ( $384 \pm 19.4$  ng/mL) compared to SAF ( $532 \pm 42.0$  ng/mL) (Fig. 4a). That in PAF ( $75 \pm 14.3$  ng/mL) and PFD ( $35 \pm 10.3$  ng/mL) was almost depleted compared to SAF and SFD (Fig. 4a). It also significantly decreased in PFD compared to PAF (Fig. 4a). Plasma leptin level in SFD ( $1.5 \pm 0.1$  ng/mL) and PFD ( $1.7 \pm 0.1$  ng/mL) significantly decreased compared to SAF ( $3.9 \pm 0.3$  ng/mL) and PAF ( $3.4 \pm 0.5$  ng/mL) (Fig. 4b). No statistical differences were observed between SAF and PAF or SFD and PFD. Plasma desacyl ghrelin in SFD ( $793.3 \pm 83.0$  fmol/mL) and PFD ( $751.6 \pm 72.0$  fmol/mL) significantly increased compared to those of SAF ( $213.3 \pm 32.9$  fmol/mL) and PAF ( $344 \pm 48.6$  fmol/mL) (Fig. 4c). There were statistical differences between SAF and PAF (Fig. 4c). Plasma active ghrelin increased in SFD ( $20.2 \pm 6.2$  fmol/mL) compared to SAF ( $5.3 \pm 0.6$  fmol/mL) (Fig. 4d). Those in PAF ( $53.0 \pm 11.6$  fmol/mL) and PFD ( $65.8 \pm 5.8$  fmol/mL) significantly increased compared to SAF and SFD (Fig. 4d). The ratio of plasma active/desacyl ghrelin  $\times 10^2$  in PAF ( $15.3 \pm 1.9$ ) and PFD ( $9.1 \pm 0.9$ ) significantly increased compared to those in SAF ( $2.6 \pm 0.3$ ) and SFD ( $2.5 \pm 0.6$ ) (Fig. 4e). There were statistical differences between PAF and PFD (Fig. 4e).

#### Feeding-regulating peptides in the SON and the PVN

The feeding-regulating peptides in the SON and the PVN were measured by ISH followed by quantification using MCID. The gene expression of the *oxytocin* in the SON and the PVN was comparable among all the experimental groups (Fig. 5a A-a–A-d, B-a–B-d, b). The gene expression of the *CRH* and the *TRH* in the PVN in SFD (Fig. 5a C-b, D-b, b) and PFD (Fig. 5a D-b, D-d, b) decreased significantly compared to those in SAF (Fig. 5a C-a, D-a,



**Fig. 4** Plasma concentrations of 5-HT, leptin, and active/desacyl ghrelin ratio Plasma levels of 5-HT (a), leptin (b), desacyl ghrelin (c), active ghrelin (d), and the ration of active/desacyl ghrelin (e) are

shown. Data are presented as mean  $\pm$  SEM. \*\* $P < 0.01$  vs. SAF, \*\*\* $P < 0.001$  vs. SAF and SFD, # $P < 0.05$ , ## $P < 0.01$  vs. PAF, ### $P < 0.001$  vs. SAF and SFD, ++ $P < 0.01$  vs. SFD

b) and PAF (Fig. 5a C–c, D-c, b). There were no statistical differences between SAF and PAF, or between SFD and PFD in the gene expression of the *oxytocin*, *CRH*, and *TRH*.

Feeding-regulating peptides in the ARC and the LHA

The feeding-regulating peptides in the ARC and the LHA were measured by ISH followed by quantification using MCID. The gene expression of the *POMC* and the *CART* in the ARC in SFD (Fig. 6a A-b, B-b, and b) decreased significantly compared to SAF (Fig. 6a A-a, B-a, b), whereas those in PFD (Fig. 6a A-d, B-d, b) were comparable to PAF (Fig. 6a A-c, B-c, b). The gene expression of the *NPY* and *AgRP* in the ARC in SFD (Fig. 6a C-b, D-b, b) and PFD (Fig. 6a C-d, D-d, b) increased significantly compared to SAF (Fig. 6a C-a, D-a, b) and PAF (Fig. 6a C-c, D-c, b). With regard to the gene expression of the *AgRP*, there were no significant differences between SFD (Fig. 6a D-b, b) and PFD (Fig. 6a D-d, b). However, the gene expression of the *NPY* in SFD (Fig. 6a C-b, b) and PFD (Fig. 6a C-d, b) differed significantly.

The gene expression of the *MCH* in the LHA in SFD (Fig. 6a E-b, b) and PFD (Fig. 6a E-d, b) increased significantly compared to those in SAF (Fig. 6a E-a, b) and

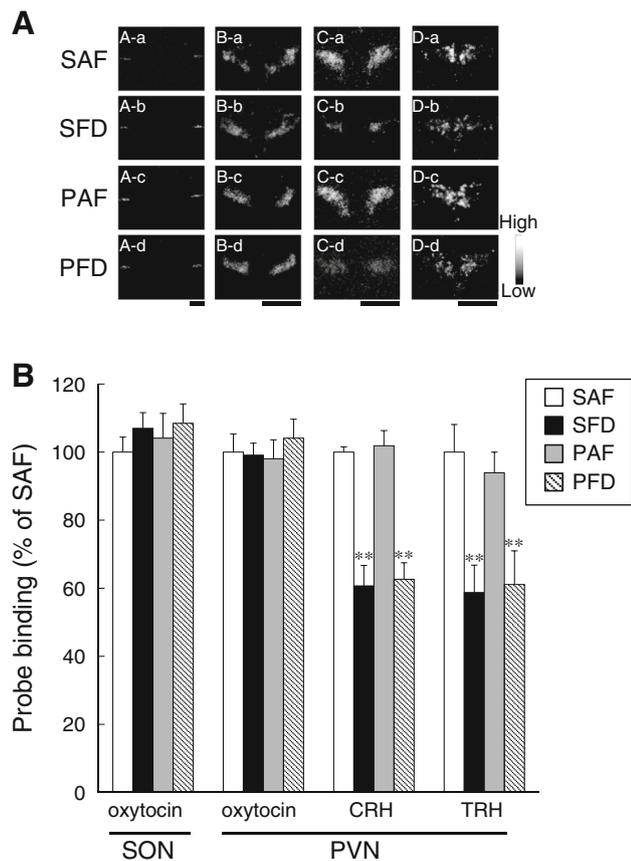
PAF (Fig. 6a E-c, b). The gene expression of the *MCH* did not differ between SFD and PFD.

The gene expression of the *orexin* was comparable among all the experimental groups (Fig. 6a F-a–F-d, b). No significant differences were observed between SAF and PAF in the gene expression of the *POMC*, *CART*, *NPY*, *AgRP*, *MCH*, and *orexin*.

Discussion

The present study comprehensively showed the relationships between the serotonergic system and feeding-regulating peptides in the hypothalamus. These results imply that the gene expression of the *POMC*, *CART* and *NPY* in the ARC may be involved in the serotonergic system in the hypothalamus.

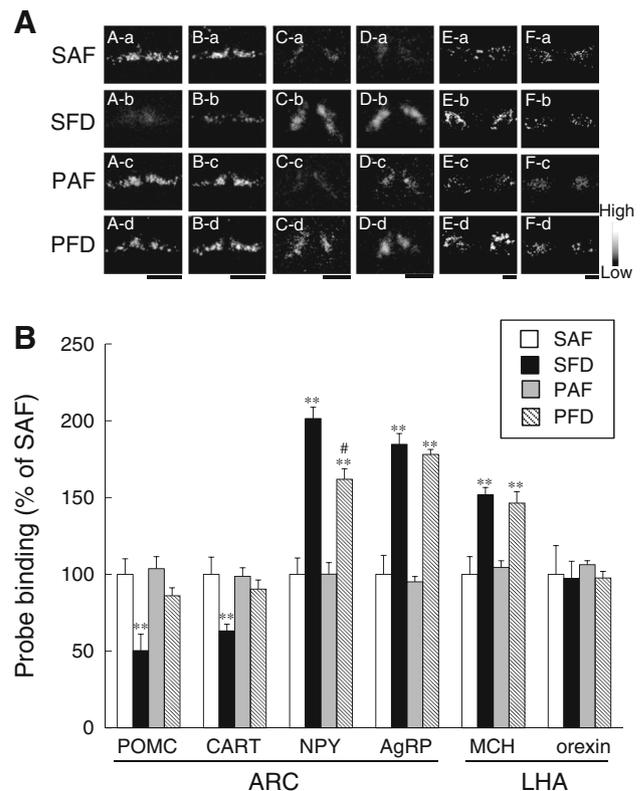
After the PCPA treatment, hypothalamic contents of NA, DA, and 5-HT were measured by HPLC to confirm the depletion of 5-HT after the treatment. As shown in Fig. 1, although NA and DA did not change with or without PCPA, 5-HT level nearly depleted after PCPA treatment, which was consistent with a previous report [10]. We made sure that the 5-HT level in the hypothalamus definitely depleted after the PCPA treatment.



**Fig. 5** Feeding-regulating peptides in the SON and the PVN **a** Digital images of ISH of *oxytocin* in the SON (A-a–A-d) and the PVN (B-a–B-d), *CRH* in the PVN (C-a–C-d), *TRH* in the PVN (D-a–D-d). Scale bar 1 mm. **b** Quantification of mRNA signals using MCID. PAF data are presented as 100 %. Data are presented as mean ± SEM. \*\**P* < 0.01 vs. SAF and PAF

After PCPA systemic treatment, the body weight decreased in PFD and PAF (Fig. 2) compared to SAF. Although the body weight in PAF after day 3 decreased significantly compared to SAF, food intakes after day 3 were comparable to SAF; thus, it was expected that there would be no significant effects on the gene expression of the feeding-regulating neuropeptides in the hypothalamus in our experimental situation after day 3.

Cumulative food intake in PFD decreased at days 4, 5, and 6 compared to those in SFD (Fig. 3). After food deprivation in the PCPA-treated group, the gene expression of the *POMC*, *CART*, and *NPY* seemed likely to be involved in 5-HT system in the hypothalamus according to our results of ISH which will be described later. Although this cumulative food intake data appear to suggest that serotonin facilitates food intake which possibly contradicts the previous concept that serotonin inhibits food intake, the body weights themselves also decreased after serotonin depletion. The decrease of body weights may be caused by complex factors, not just by decreased food intake. Thus,



**Fig. 6** Feeding-regulating peptides in the ARC and the LHA **a** Digital images of ISH of *POMC* in the ARC (A-a–A-d), *CART* in the ARC (B-a–B-d), *NPY* in the ARC (C-a–C-d), *AgRP* in the ARC (D-a–D-d), *MCH* in the LHA (E-a–E-d), and *orexin* in the LHA (F-a–F-d). Scale bar 1 mm. **b** Quantification of mRNA signals using MCID. PAF data are presented as 100 %. Data are presented as mean ± SEM. #*P* < 0.05 vs. SFD, \*\**P* < 0.01 vs. SAF and PAF

we decided to evaluate accumulating food intake but not food intake per body weight. This analysis appears on the surface to produce opposite results when the food intake is expressed as per body weight, but in fact complex factors may be involved in decreased food intake in each animal after 5-HT-depletion. This point should be examined by further study. We have not examined the gene expression of the feeding-regulating peptides in the hypothalamus after re-feeding; however, it is speculated that some of the feeding-regulating peptides in the hypothalamus may not change despite re-feeding with PCPA treatment.

The significant decrease in PG in SFD and PFD compared to that in SAF and PAF might have been due to the effect of food deprivation. No statistical differences were seen between SAF and PAF or between SFD and PFD, and P-Osm did not differ among all the experimental groups, suggesting that we can compare the feeding-regulating neuropeptides in the hypothalamus in this experiment. Plasma 5-HT dramatically decreased after PCPA treatment (Fig. 4a), but not as completely as that of the hypothalamus. And it tended to decrease with food deprivation.

These result suggests that peripheral 5-HT was also degraded by PCPA as was hypothalamic 5-HT. As previously described [23, 24], food restriction decreased 5-HT levels, which is consistent with our results. Because leptin is a satiety molecule [25, 26], it significantly decreased in food deprivation. But there were no statistical changes between SAF and PAF or SFD and PFD, suggesting that PCPA treatment did not affect plasma leptin levels.

Food deprivation for 48 h significantly increased plasma desacyl ghrelin concentration, indicating the adaptive response to starvation [27]. This was the case in plasma-active ghrelin in SFD; however, it significantly increased in PAF and PFD. These results may indicate that PCPA systemic treatment itself decreased food intake, which resulted in increasing active ghrelin secretion as a secondary response. One possible explanation is that a large amount of active ghrelin had already been secreted by the PCPA treatment in PAF and additional food deprivation produced no further changes in PFD. However, it is difficult to evaluate whether 5-HT may be involved in the regulation of circulating active ghrelin from the data obtained from the present study. Further study should be carried out to confirm this and to elucidate its mechanism.

Among all the feeding-regulating neuropeptides we examined, there was no remarkable difference between SAF and PAF, which indicates that PCPA itself did not affect the peptides under the free feeding condition. Therefore, if the feeding-regulating peptide was markedly different between SFD and PFD, it may be said that the peptide is regulated via the serotonergic system in the hypothalamus.

The gene expression of the *oxytocin*, which is recognized as an anorexic peptide [28], in the SON and the PVN, did not differ despite food deprivation (Fig. 5a, b). It is considered that no significant difference can be detected as a state of *oxytocin* mRNA. Further study, such as a comparison with heteronuclear RNA expression [29], is needed.

The gene expression of the *CRH* and the *TRH* in the PVN decreased significantly after 48 h food deprivation (Fig. 5a, b), as previously described [9, 11–13]. They also decreased after food deprivation under the PCPA pretreatment, which suggests that 5-HT in the brain does not affect the gene expression of *CRH* and *TRH*. Although it has been described that 5-HT 2C receptors exists in the CRH neuron and that there is a possible interaction between 5-HT and CRH [30], our experiment was performed under a condition of almost completely depleted 5-HT, and we examined only one point, so a simple comparison cannot be made. The time course of the gene expression of the *CRH* may have gradually changed after the start of food deprivation.

As expected, the gene expression of the *POMC* and *CART* decreased significantly after food deprivation (Fig. 6a, b). Strikingly, however, they remained unchanged by PCPA pretreatment despite food deprivation. Thus, the effects of PCPA are not statistically confirmed. *CART* in the ARC is co-stored with *POMC* [31] and 5-HT receptors exist in the *POMC* neuron in the ARC [17, 32]. Our results indicate that the serotonergic system in the hypothalamus may project to the *POMC* and *CART* neurons in the ARC, which is consistent with previous reports [17, 33].

The gene expression of the *NPY* in the ARC increased significantly after food deprivation (Fig. 6a, b). While the gene expression of the *NPY* also increased significantly after food deprivation with PCPA pretreatment, the extent of the rate of increase was not uniform. The gene expression of the *NPY* in PFD did not increase adequately compared to SFD. As the *NPY* neurons in the ARC are also expressed in 5-HT receptors [34], there may have been a possible interaction with 5-HT. Taken together with our results, 5-HT may be partially related to *NPY* regulation.

As previously reported [15], the gene expression of the *MCH* increased significantly after food deprivation (Fig. 6a, b). The variation was to the same degree as with PCPA pretreatment. Our results indicate that 5-HT had no effect to the gene expression of the *MCH*. As with the result of *oxytocin*, the gene expression of the *orexin* did not differ despite food deprivation, which indicates that 5-HT in the hypothalamus may not affect the gene expression of the *MCH*.

In conclusion, we have shown that there may have been possible interaction between the serotonergic system in the hypothalamus and the gene expression of the *POMC*, *CART*, and *NPY* in the ARC. These findings provide evidence of the mechanism of the neural circuit that mediates feeding-regulating neuropeptide reaction in response to 5-HT in the hypothalamus.

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**Conflict of interest** All authors declare that they have no conflict of interest.

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