Brain-derived neurotrophic factor (BDNF) has been implicated in the regulation of development, survival, differentiation and synaptic plasticity in the nervous system (1). In addition to these roles, BDNF has been demonstrated to be an anorectic factor. Heterozygous BDNF mutant mice and brain-specific BDNF knockout mice, in which the BDNF gene has been selectively deleted in the brain after birth, showed increased food intake and body weight (2–4). Intracerebroventricular BDNF infusion markedly reduced appetite and body weight in rats (5). Furthermore, BDNF alleviated obesity and hyperglycaemia in several obesity models (6, 7).

TrkB is a high-affinity receptor for BDNF. BDNF activates the TrkB tyrosine kinase receptor, followed by phosphorylation of extracellular signal-regulated kinase. Mouse mutants that express TrkB in the brain at approximately one-quarter of the normal level show hyperphagia and excessive weight gain on high-fat diets (8). In human studies, patients with BDNF haploinsufficiency displayed

hyperphagia, severe obesity, hyperactivity and impaired cognitive function (9). Additionally, patients with a mutation in TrkB were found to be hyperphagic and obese (10). Taken together, these reports suggest that BDNF-TrkB signalling plays an important role in the regulation of feeding behaviours and body weight under both physiological and pathophysiological conditions.

Wong and colleagues recently demonstrated that a single injection of BDNF into the paraventricular nucleus of the hypothalamus (PVN) reduced energy intake (11) and increased energy expenditure (12), thus indicating that the PVN is an important target for BDNF action. However, the specific cell types in the PVN that regulate BDNF-mediated effects on feeding behaviour remain to be elucidated.

Corticotrophin-releasing hormone (CRH), a major stress response hormone, potently inhibits food intake and stimulates energy expenditure via the sympathetic nervous system (13). Urocortin, a member of the CRH family, decreases food intake, increases energy expenditure and attenuates body weight gain more potently than CRH (14). It has recently been shown that both single and chronic BDNF administration increases CRH levels in the hypothalamus, particularly in the PVN (15, 16). Therefore, we hypothesised that the CRH system mediates BDNF-effects on feeding and energy expenditure.

In the present study, BDNF was continuously infused i.c.v. over an extended time, using an osmotic mini-pump, to evaluate the chronic effects of BDNF. The roles of CRH and CRH receptors were assessed using specific antagonists. In addition, pair-feeding and calorimetry were used to measure food intake and energy expenditure and to assess their individual impacts on body weight.

It is shown that i.c.v. infusion of BDNF increased CRH and urocortin expression in the PVN and that local infusion of BDNF into the PVN markedly decreased food intake. Furthermore, chronic i.c.v. administration of BDNF induced anorexia and lipolysis, mediated primarily by the CRH/urocortin system.

Materials and methods

Materials

Recombinant human BDNF was supplied by Dainippon Sumitomo Pharma Co., Ltd. (Osaka, Japan).

Animals

Mature male Wistar rats (Std : Wistar), 10–11 weeks of age (Japan SLC Ltd., Tokyo, Japan), were housed in a room under a 12 : 12 h light/dark (lights on 07.30 h) at a temperature of 24 °C and 55% humidity. The rats were allowed free access to standard rat chow (CE-2; Clea, Osaka, Japan) and water. All procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the Jichi Medical University and were performed in accordance with the Japanese Physiological Society's guidelines for animal care.

Local and i.c.v. infusion of BDNF

Male rats were anesthetised by i.p. injection of avertin (tribromoethanol, 200 mg/kg) and placed in a stereotaxic frame. For chronic infusion, an osmotic mini-pump (model 1002, 0.25 μl/h for 14 day; Alzet, Cupertino, CA, USA) was filled immediately prior to implantation, with either BDNF (2.5 μg/μl for 15 μg/day treatment) in artificial cerebrospinal fluid (aCSF) or aCSF alone. aCSF contained (in mM); 296.4 mM NaCl, 6 mM KCl, 2.8 mM $CaCl_2{\cdot}H_2O$, 1.6 mM $MgCl_2$, 1.6 mM Na_2HPO_4 and 0.4 mM $NaH_2PO_4{\cdot}H_2O$ at pH 7.4. In some experiments, the CRH antagonist α-helical-$CRH_{9\text{-}41}$ (0.16 μg/μl for 0.96 μg/day; C246; Sigma Aldrich, St Louis, MO, USA), the CRH-receptor 1 (R1) antagonist, antalarmin hydrochloride (1.67 μg/μl for approximately 10 μg/day treatment; Sigma-Aldrich), and the CRH-receptor2 (R2), anti-sauvagine 30 (anti-SV30) (0.5 μg/μl for approximately 3 μg/day treatment; Phoenix Pharmaceuticals, Burlingame, CA, USA) were injected with or without BDNF (15 μg/day treatment). Anti-SV30 was diluted in aCSF containing 80% dimethyl sulphoxide (DMSO) and its control solution also contained 80% DMSO. The osmotic mini-pump was then connected to a cannula using 5 cm of vinyl tubing (Brain infusion kit; Alzet), and the cannula was stereotactically placed into the brain, with the tip in the left lateral ventricle (coordinates: 0.8 mm posterior to bregma, 1.5 mm lateral to the midline and 3.5 mm below the skull surface). The osmotic mini-pump was implanted subcutaneously over the scapula. For PVN chronic infusion, the solutions described above were diluted ten-fold with aCSF, and the cannula (30-gauge dental injection needle) was stereotactically placed into the PVN (coordinates: 1.8 mm caudal to bregma, 0.5 mm lateral to the midline and 7.4 mm below the skull surface).

Measurements of food intake and body weight combined with pair-feeding

Food intake over 24 h was calculated by weighing the remaining food pellets, and body weight was measured between 17.30 h and 18.30 h every day. For pair-feeding, the amount of food consumed by the BDNF-treated group over the course of 24 h was measured at 17.30 h, and a corresponding amount of pellets was given to the pair-fed group over a 24-h period.

Real-time polymerase chain reaction (PCR) analysis

Brains were isolated from rats and frozen on dry ice for a few minutes. Brain slices that contained the entire PVN (excised from the left and right sides) were prepared using rat brain matrices (Tedpella, Redding, CA, USA). Total RNA was isolated from the PVN using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and was treated with RQ1-DNase (Promega, Madison, WI, USA) to remove residual DNA contamination. First-strand cDNA synthesis was performed using Superscript III (Invitrogen). Primers for real-time PCR were initially examined for correct product size and the absence of primer-dimer formation by using HotStarTaq DNA polymerase (Qiagen, Hilden, Germany) for 30 cycles of 15 s at 94 °C, 20 s at 60 °C and 20 s at 72 °C, followed by agarose gel electrophoresis. The cDNA was analysed by quantitative real-time PCR using a Thermal Cycler Dice Real Time System (TP800; Takara, Tokyo, Japan). Each real-time PCR was performed in a total reaction volume of 25 μl containing cDNA synthesised from 50 ng of total RNA, 0.2 M of each primer, and SYBER Premix Ex Taq (Takara), with an initial denaturation for 10 s at 95 °C, followed by 40 cycles of 5 s at 95 °C and 30 s at 60 °C. All real-time PCR experiments were performed in quadruplicate. Different cDNA samples were normalised to a housekeeping gene, glyceraldehyde 3-phosphate dehydrogenase (G3PDH). The primers used for PCR were: for CRH, 5′-TCTCTCTGGATCTCACCTTCCACC-3′ and 5′-AGCTTGCTGAGCTAACTGCTCTGC-3′; for urocortin, 5′-TTGCACTGGATAGACACTCCGATAA-3′ and 5′-TTCGGATCCTGGACCACATTC-3′; and for G3PDH, 5′-GGCACAGTCAAGGCTGAGAATG-3′ and 5′-ATGGTGGTGAAGACGCCAGTA-3′.

Double immunohistochemistry of CRH and TrkB in PVN

Cannulae were implanted into the rat lateral ventricle. One week after implantation, rats were i.c.v injected with 10 μl of colchicine solution

(20 $\mu g/\mu l$ in saline, 039-03851; Wako Pure Chemical Industries, Osaka, Japan). Twenty-four hours after injection, rats were perfused transcardially with 200 ml of 4% paraformaldehyde/0.2% picric acid in 0.1 M phosphate buffer (PB) at pH 7.4, followed by 100 ml of saline under deep urethane anaesthesia. Brains were removed, postfixed by the same fixative for 1 day, and cryoprotected with 30% sucrose in 0.1 M PB for 2 days at 4 °C. Brains were sectioned coronally at 40-μm thickness with a freezing microtome. Sections were treated simultaneously with goat anti-TrkB (1:25, SC-12; Santa Cruz Biotechnology, CA, USA) and rabbit anti-CRH (1 : 1000, T-4037; Peninsula Laboratories, CA, USA) in 0.1 M phosphate-buffered saline (PBS) containing 0.5% Triton-X and 5% skim-milk for 1 day at 4 °C after incubation with same solution except antibodies for 1 h at room temperature to block nonspecific binding of antibodies. After washing with PBS, sections were treated sequentially with AlexaFluor568-labelled donkey anti-goat IgG and AlexaFluor488-labelled goat anti-rabbit IgG (dilution 1 : 200; Invitrogen) in the same solution used for the first antibodies each for 2 h at room temperature. After washing again, sections were mounted on glass slides with VectaSield (VectaLab, Burlingame, CA, USA) and observed with a confocal microscope (Fluoview FV1000; Olympus, Tokyo, Japan).

Measurement of serum corticosterone

Blood samples were immediately collected by decapitation. To collect plasma, one-tenth volume of 60–70 KIU/ml Aprotinin (012-18114; Wako) and 0.1 M ethylenediaminetetraacetic acid were added. Samples were centrifuged at 4 °C and plasma was stored at −80 °C until assayed for corticosterone levels. Plasma corticosterone was measured using AssayMax Corticosterone enzyme-linked immunosorbent assay kit (Assaypro, St Charles, MO, USA).

Adipose tissue weight and serum triglyceride level

At day 12 after chronic infusion, serum was rapidly collected, and adipose tissues were dissected and weighted. The triglyceride level was assessed using a triglyceride E test kit (Wako).

Histological analysis of fat tissues

Small pieces of epididymal white adipose tissue (WAT) were dissected, washed in saline, fixed with 4% paraformaldehyde, and embedded in paraffin. Tissue sections were cut at a thickness of 15 μm and stained with hematoxylin. To examine the sizes of the white and brown adipocytes, the adipocyte number was counted in six appropriate, limited areas (WAT; $25 \times 10^{-3}/mm^2$) of each stained specimen ($n = 5$). Multilocular adipocytes in the section were not counted.

Indirect calorimetry

The energy expenditure was measured by indirect calorimetry using an indirect open-circuit calorimeter (Oxymax; Columbus Instruments, Columbus, OH, USA). After the system was calibrated against standard gas mixtures, rats were placed in individual acrylic calorimeter chambers, with free access to food and water. The energy expenditure, defined as oxygen consumption (VO_2), and the carbon dioxide production (VCO_2) were measured during a 20-h period (from 13.00 h to 09.00 h) at room temperature. Pairwise measurements were taken. Expired air was analysed for a 60-s period every 16 min, using an electrochemical oxygen analyser and a carbon dioxide sensor (Oxymax). The respiratory exchange ratio was calculated as the volume of CO_2 produced (VCO_2) per volume of O_2 consumed (VO_2), in ml/min.

Statistical analysis

All data are expressed as the mean ± SEM. For statistical analyses, one-way ANOVA followed by Tukey's test or Student's t-test was used, as appropriate, to determine differences between individual means. $P < 0.05$ was considered statistically significant.

Results

BDNF-induced increase in the expression of CRH and urocortin in the PVN. It is unknown whether BDNF acts via CRH in the PVN to produce anorectic effects, and whether BDNF increases the expression of urocortin, a member of the CRH family. We examined the alterations in CRH and urocortin gene expression after a 12-day i.c.v. infusion of BDNF. Real-time PCR showed that i.c.v. administration of BDNF for 12 days induced an 18.2-fold increase in CRH mRNA (Fig. 1A) and a 3.4-fold increase in urocortin mRNA in the PVN (Fig. 1B).

To examine whether BDNF could directly interact with CRH neurones in the PVN, we measured localisation of the BDNF receptor TrkB and CRH in the PVN by double immunohistochemistry. In rats pretreated with colchicine, most of the PVN CRH-immunoreactive neurones expressed TrkB proteins (Fig. 1C–H).

The effects of i.c.v. infused BDNF and α-helical-CRH_{9-41} on food intake and body weight

We examined the involvement of the endogenous CRH pathway in the effects of BDNF on food intake. BDNF was continuously administered i.c.v. with or without α-helical-CRH_{9-41} for 12 days. BDNF suppressed the food intake and body weight gain, and α-helical-CRH_{9-41} attenuated the effects of BDNF (Fig. 2A,B). The body weight gain of BDNF-treated rats was comparable to that of untreated rats fed an equivalent amount of food. α-helical-CRH_{9-41} alone had no significant effect on food intake or body weight in any of the experiments.

The effects of BDNF and α-helical-CRH_{9-41} locally infused into the PVN on food intake and body weight

To study whether the anorexigenic and weight-reducing effects of i.c.v. BDNF are produced through its action on the PVN, the effects of BDNF focally administered to the PVN was examined. Local administration of BDNF into the PVN significantly decreased both food intake and body weight to the levels similar to those observed with i.c.v. administration of BDNF (Fig. 3A,B), and these effects were significantly counteracted by local infusion of α-helical-CRH_{9-41} (Fig. 3A,B). α-helical-CRH_{9-41} alone had no significant effect on food intake (at day 10 of treatment: 122.1 ± 10.9 for control versus 133.3 ± 1.9 g for α-helical-CRH_{9-41}, not significant) and body weight (at day 10 of treatment: 12.4 ± 3.4 g for control versus 8.5 ± 5.3 for α-helical-CRH_{9-41}, not significant).

Next, we examined the alteration in CRH gene expression in the PVN at 12-day of local infusion of BDNF into the PVN. Real-time PCR showed that the CRH mRNA level in the PVN was significantly increased approximately threefold by BDNF compared to

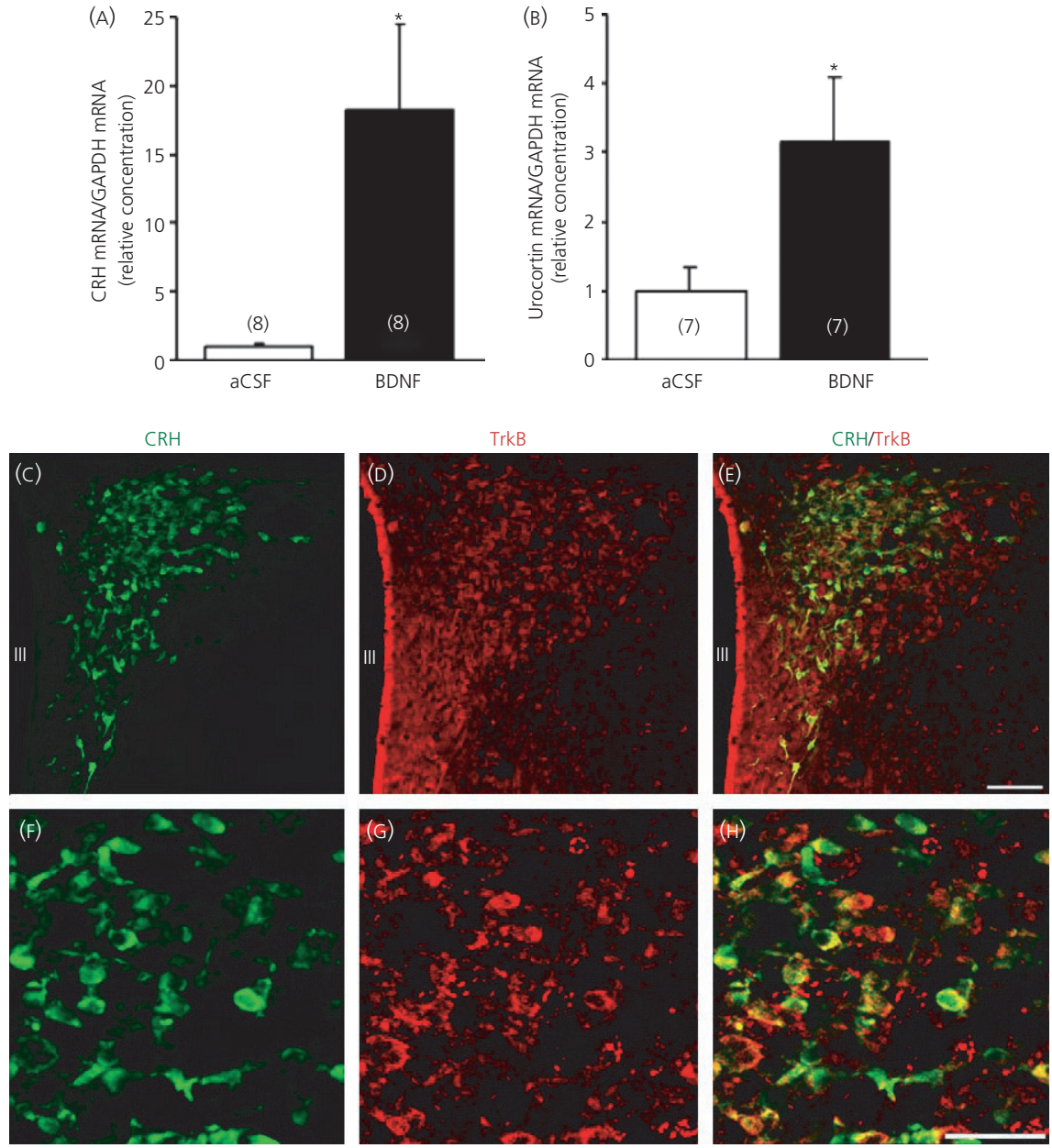

Fig. 1. Brain-derived neurotrophic factor (BDNF) increased the expression of corticotrophin-releasing hormone (CRH) and urocortin mRNA in the paraventricular nucleus (PVN). (A, B) Expression of CRH (A) and urocortin (B) mRNA in the PVN, based on real-time polymerase chain reaction (n = 7 for aCSF and BDNF groups). *P<0.05 versus aCSF. (C–H) CRH (C and F; green) and TrkB (D, G; red) were expressed in the PVN neurones. CRH-expressing neurones co-expressed TrkB (E, H; merge, yellow). III represents the third ventricle in (C–E). (F–H) Higher magnifications of (C–E). Scale bar = 100 μm for (C–E), 50 μm for (F–H).

aCSF control (relative quantity: 0.0128 ± 0.0026, n = 4 for BDNF versus 0.0039 ± 0.0016, n = 4 for control, P < 0.05).

Furthermore, we examined plasma corticosterone level, which is under control of CRH and hypothalamus-pituitary-adrenal (HPA) axis. In this study, however, the plasma corticosterone level in BDNF-treated animals (14.3 ± 5.3 ng/ml, n = 4) was not significantly different from that in CSF-treated control animals (12.2 ± 2.7 ng/ml, n = 4).

The effects of α-helical-CRH$_{9-41}$ on BDNF-induced changes in the adipose tissue content and serum triglyceride level

We examined the effects of BDNF and α-helical-CRH$_{9-41}$ on fat weight. After 12 days of i.c.v. infused BDNF, subcutaneous, perirenal, mesenteric and epididymal fat pad weights were all decreased. The effects of BDNF on the WAT weight at these four

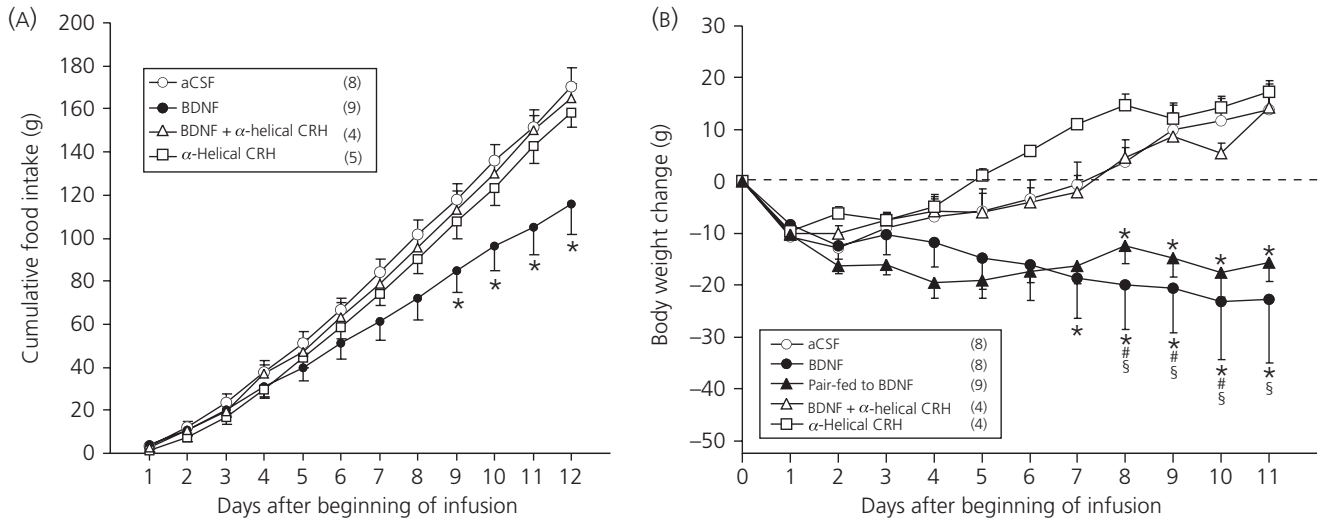

Fig. 2. The i.c.v. infusion of brain-derived neurotrophic factor (BDNF) decreased food intake and body weight, and these effects were counteracted by α-helical-corticotrophin-releasing hormone (CRH). (A) Chronic i.c.v. infusion of BDNF decreased the 24-h food intake at days 9–12 after pump implantation, and α-helical-CRH attenuated the reduction in food intake. (B) The i.c.v. infusion of BDNF decreased body weight gain at post-implant days 7–11, and α-helical-CRH reversed these reductions. Body weight gains in artificial cerebrospinal fluid (aCSF)-treated rats pair-fed to the BDNF-treated group were comparable to those in the BDNF-treated group. aCSF group, n = 8; BDNF group, n = 9; pair-fed group, n = 6; BDNF + α-helical-CRH group, n = 4; α-helical-CRH group, n = 5. *P < 0.05 versus aCSF, #P < 0.05 versus BDNF + α-helical-CRH, §P < 0.05 versus α-helical-CRH.

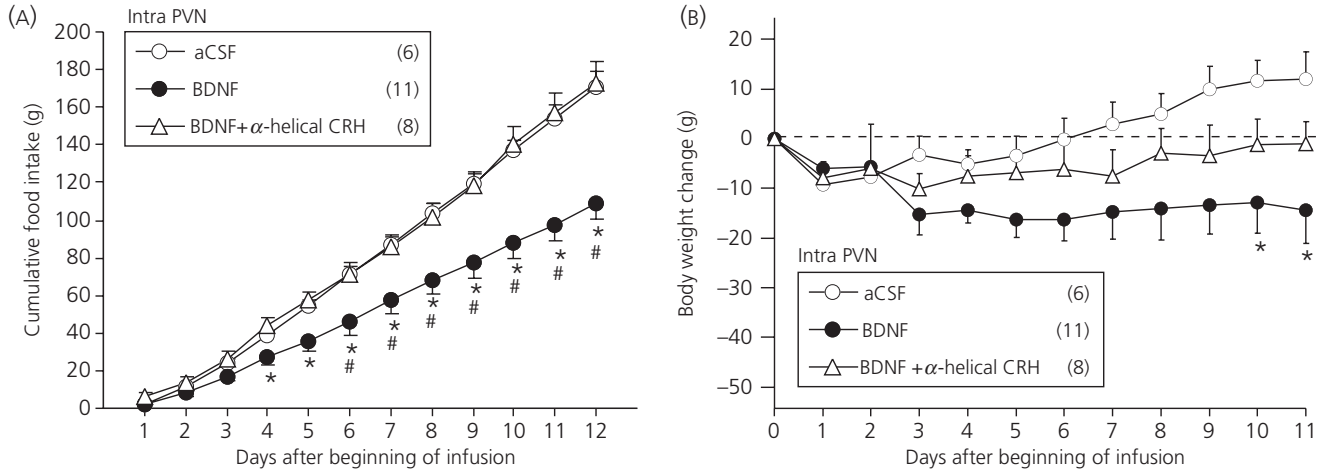

Fig. 3. Intra-paraventricular nucleus (PVN) brain-derived neurotrophic factor (BDNF) infusion decreased food intake and body weight, and these effects were counteracted by α-helical-corticotrophin-releasing hormone (CRH). (A) Local infusion of BDNF into the PVN decreased the 24-h food intake at post-implant days 4–12, and α-helical-CRH inhibited the BDNF-mediated effect on food intake at days 6–12. (B) Local infusion of BDNF into the PVN decreased body weight gain at post-implant days 10–11, and α-helical-CRH attenuated the reduction in body weight. Artificial cerebrospinal fluid (aCSF) group, n = 6; BDNF group, n = 11; BDNF + α-helical-CRH group, n = 8. *P < 0.05 versus aCSF, #P < 0.05 versus BDNF + α-helical-CRH.

distinct locations were reversed by α-helical-CRH_{9-41} (Fig. 4A). BDNF significantly decreased the serum triglyceride level, and α-helical-CRH_{9-41} counteracted this effect (Fig. 4B). In addition, BDNF reduced WAT cell size compared to the aCSF control (Fig. 4C), and the effect on adipocytes was attenuated by α-helical-CRH_{9-41}.

The effects of α-helical-CRH_{9-41} on the respiratory quotient and rectal temperature

To examine the effects of BDNF on energy expenditure, we evaluated VO_2 and VCO_2 and the respiratory quotient (RQ), which reflects glucose and lipid metabolism *in vivo*. In addition, to exclude possible secondary effects as a result of altered food intake, the average amount of food consumed by the BDNF-treated rats was given to a group of untreated rats (pair-fed group). The VO_2 (Fig. 5A) and VCO_2 (Fig. 5B) in BDNF-treated rats were comparable to those in aCSF-treated rats, α-helical-CRH_{9-41} and BDNF-treated rats and pair-fed rats at 2 weeks after chronic i.c.v. administration of BDNF. The RQ (VCO_2/VO_2) in BDNF-treated rats was significantly lower than the RQs in aCSF-treated control rats and in pair-fed rats (Fig. 5C). α-helical-CRH_{9-41} blocked the suppression of RQ (Fig. 5C) caused by BDNF.

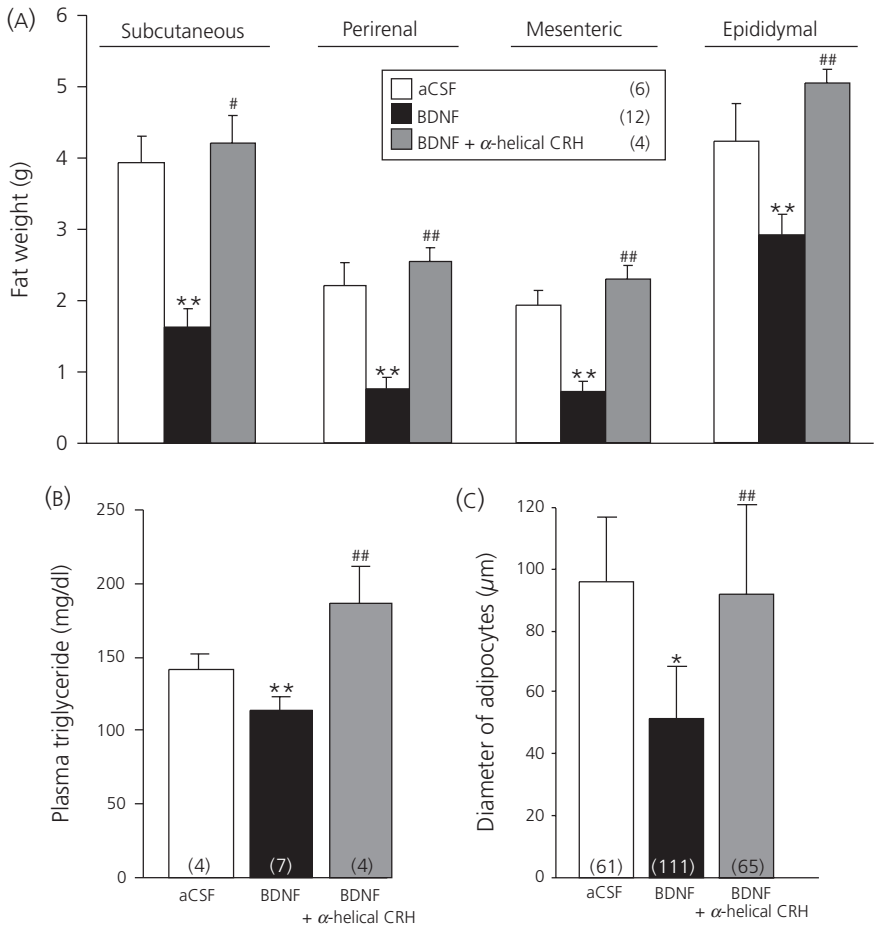

Fig. 4. The i.c.v. infusion of brain-derived neurotrophic factor (BDNF) decreased subcutaneous and visceral fad pads, plasma triglycerides and adipocyte size. These effects were counteracted by α-helical-corticotrophin-releasing hormone (CRH). (A) BDNF decreased subcutaneous, perirenal, mesenteric and epididymal fat pad weights, and α-helical-CRH attenuated the BDNF-induced reductions. Artificial cerebrospinal fluid (aCSF) group, n = 6; BDNF group, n = 12; BDNF + α-helical-CRH group, n = 4. (B) BDNF reduced the plasma triglyceride level compared to the aCSF control level, and this was reversed by α-helical-CRH. aCSF group, n = 4; BDNF group, n = 7; BDNF + α-helical-CRH group, n = 4. (C) BDNF decreased adipocyte diameter, and this was reversed by α-helical-CRH. aCSF group, n = 61 cells; BDNF group, n = 111 cells; BDNF + α-helical-CRH, n = 65 cells (n = 5 animals in each group). *P < 0.05 versus aCSF, **P < 0.01 versus aCSF, #P < 0.05 versus.BDNF and ##P < 0.01 versus BDNF.

At 2 weeks after injection, the BDNF-treated group also showed an increase in rectal temperature (Fig. 5D) compared to both aCSF-treated and pair-fed groups, The BDNF-induced elevation in body temperature was attenuated by α-helical-CRH_{9-41}, indicating that BDNF increased body temperature independently of food intake and via a CRH-mediated mechanism.

Effects of antagonists for CRH-R1 and R2 on food intake and body weight

To estimate the receptor subtypes that mediate the effect of BDNF on feeding and energy expenditure, CRH-R1 antagonist, antalarmin, and CRH-R2 antagonist, anti-SV30, were examined. Antalarmin showed little effects on the decreases of food intake and body weight caused by BDNF (Fig. 6A, B). Anti-SV30 tended to attenuate the anorectic effect of BDNF, although it was not significant (Fig. 6C). Anti-SV30 significantly (P < 0.01–0.05) recovered the body weight reduced by BDNF from day 5 to day 11 (Fig. 6D).

Discussion

Chronic i.c.v. infusion of BDNF via an osmotic mini-pump increased the expression of CRH and urocortin in the PVN and induced a reduction in food intake and body weight. The i.c.v. administration of a CRH receptor antagonist, α-helical-CRH_{9-41}, attenuated the anorectic and body weight reducing effects of BDNF. These results indicate that i.c.v. infused BDNF inhibits feeding primarily through CRH and urocortin pathways.

Local infusion of BDNF into the PVN markedly decreased food intake and body weight. These effects of locally infused BDNF were attenuated by local infusion of α-helical-CRH_{9-41} into the PVN, suggesting that the PVN are targeted by BDNF-evoked CRH and urocortin pathways. It has been reported that CRH suppresses feeding when it is injected into the PVN, but not the lateral hypothalamus, ventromedial hypothalamic nucleus (VMH), globus pallidus or striatum in rats (17), and that urocortin injection into the PVN suppresses feeding (18, 19), whereas no effect was reported in one

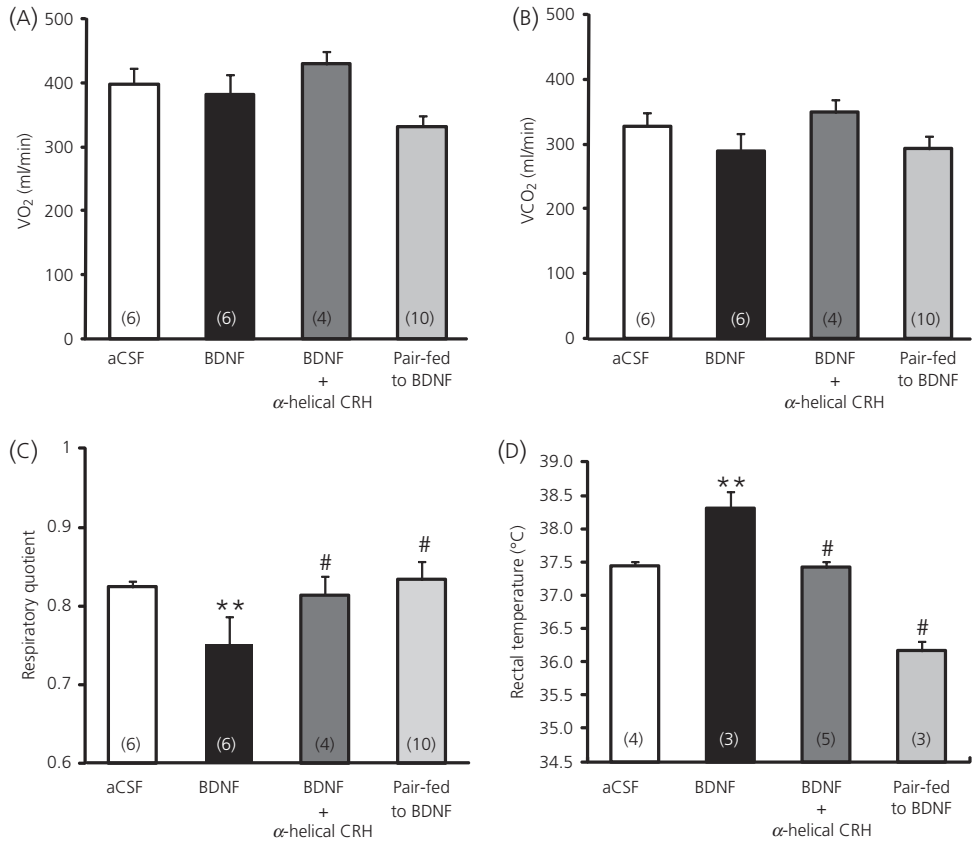

Fig. 5. The i.c.v. infusion of brain-derived neurotrophic factor (BDNF) decreased the respiratory quotient and increased body temperature, and these effects were counteracted by α-helical-corticotrophin-releasing hormone (CRH). (A) VO_2 and (B) VCO_2 in the artificial cerebrospinal fluid (aCSF)-treated, BDNF-treated, BDNF + α-helical-CRH-treated and pair-fed groups. (C) BDNF significantly decreased the respiratory quotient compared to that in the aCSF control, and this effect was counteracted by α-helical-CRH. The respiratory quotients in the aCSF-treated group and the pair-fed group were higher than that in the BDNF-treated group. aCSF group, n = 6; BDNF group, n = 6; BDNF + α-helical-CRH group, n = 4; pair-fed group, n = 10. (D) BDNF increased the body temperature, and this was counteracted by α-helical-CRH. The body temperature of the pair-fed group was lower than that of the BDNF-treated group. aCSF group, n = 4; BDNF group, n = 3; BDNF + α-helical-CRH group, n = 5; pair-fed group, n = 3. **P < 0.01 versus aCSF and #P < 0.05 versus BDNF.

study (20). Collectively, the findings of the present study suggest that both BDNF and BDNF-stimulated CRH and urocortin act on the PVN as their primary effecter site for inhibiting food intake.

BDNF is expressed abundantly in the PVN (21), VMH and dorsomedial hypothalamic nucleus (8). Hence, BDNF could be released and could regulate CRH neurones within the PVN. Alternatively, given that VMH neurones reportedly project to the PVN (22) and that VMH-selective knockdown of BDNF induces hyperphagia and obesity (23), BDNF neurones in the VMH may innervate and stimulate PVN CRH and urocortin neurones. These pathways may function when satiety centres (i.e. the PVN and VMH) are activated by food intake. BDNF neurone projection sites remain to be further elucidated.

In rodents, two CRH receptor subtypes, CRH-R1 and CRH-R2, are located in the hypothalamus (24). It is known that CRH-R1 is the high affinity receptor for both CRH and urocortin, whereas CRH-R2 has high affinity for urocortin and low affinity for CRH. Hence, urocortin more potently than CRH interacts with CRH-R2 (25). CRH-R1 and CRH-R2 are reportedly expressed in the PVN (26). We used their specific antagonists to assess the involvement of these receptors in the effect of BDNF. Antalarmin, an antagonist of CRH-R1, had little action on the effects of BDNF on feeding and body weight. Anti-SV30, an antagonist of CRH-R2, moderately attenuated the BDNF-induced anorexia and significantly recovered body weight. These data indicate that CRH-R2 receptor is involved in the action of BDNF on feeding and body weight and that urocortin plays a more crucial role than CRH in mediating the action of BDNF to decrease body weight.

The results obtained indicatinh that the effect of the CRH-R2 antagonist was lesser than that of α-helical-CRH_{9-41}, the dual antagonist, suggest that the CRH-R1 receptor pathway, albeit ineffective by itself, could cooperative with the CRH-R2 receptor pathway to fully mediate the BDNF action to regulate feeding.

However, it is difficult to quantitatively evaluate the contribution of two receptors only by the experiments with antagonists. To answer this question, it would be useful to perform region-specific gene knockout or knockdown experiments.

In our experiments, i.c.v BDNF failed to significantly alter VO_2 and VCO_2 that is unnormalised with body weight, whereas it decreased the RQ. These results suggest that BDNF does not increase energy expenditure, although it promotes the use of fat

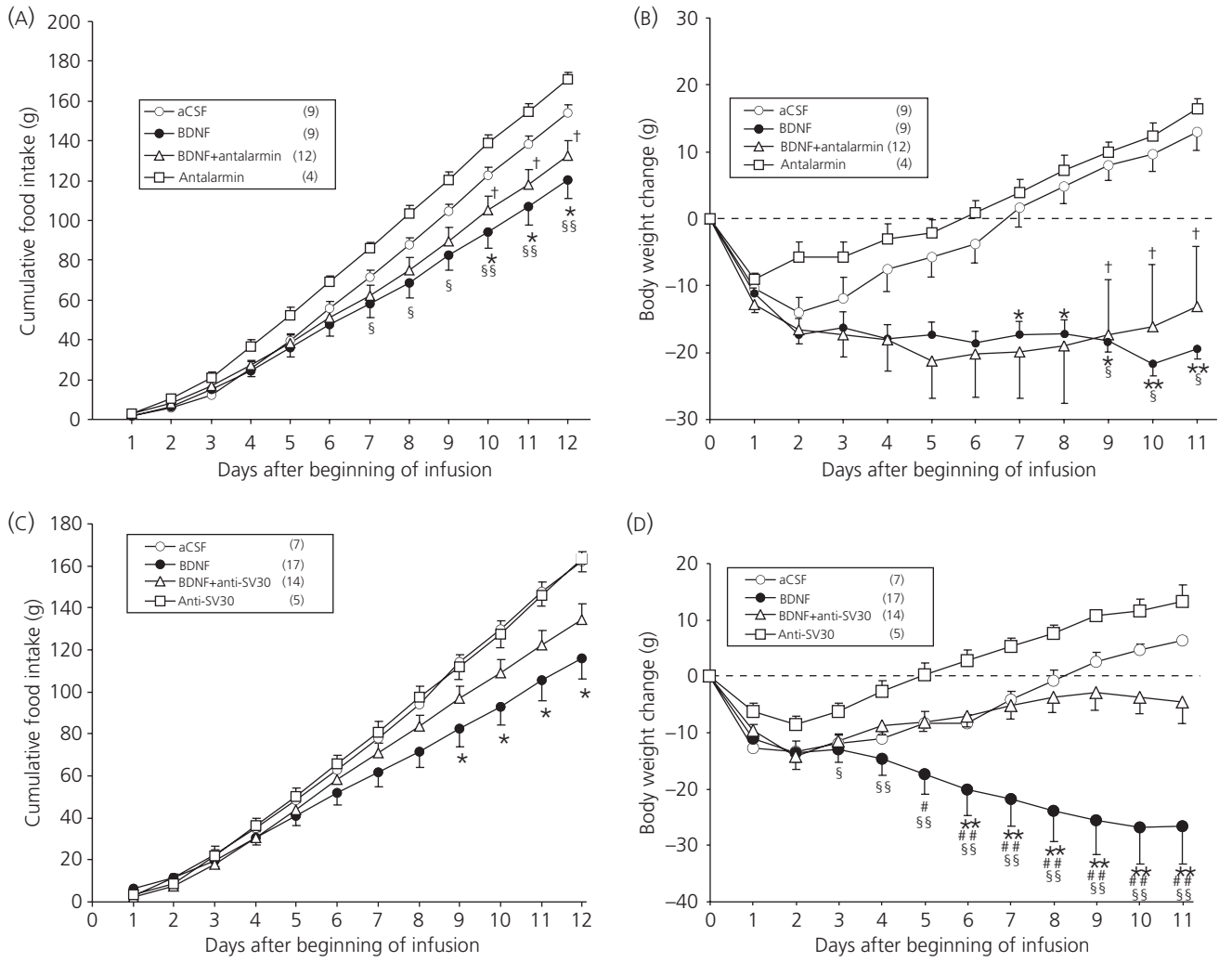

Fig. 6. Effects of corticotrophin-releasing hormone (CRH)-R1 and CRH-R2 antagonist on brain-derived neurotrophic factor (BDNF)-induced decreases in food intake and body weight. (A, B) Chronic i.c.v. infusion of BDNF decreased 24-h food intake, and the CRH-R1 antagonist, antalarmin, had little action on the effects of BDNF on feeding and body weight; n = 9 for artificial cerebrospinal fluid (aCSF), n = 9 for BDNF, n = 12 for BDNF and antalarmin, and n = 4 for antalarmin. *P < 0.05 BDNF versus aCSF, **P < 0.01 BDNF versus aCSF, §P < 0.05 BDNF versus antalarmin, §§P < 0.01 BDNF versus antalarmin, †P < 0.05 BDNF + antalarmin versus antalarmin. (C, D) CRH-R2 antagonist, anti-sauvagine 30 (anti-SV30), tended to attenuate the reduction in food intake at days 6–12 (C) and significantly reversed the reduction in body weight induced by BDNF (D) at days 5–11; n = 8 for aCSF, n = 17 for BDNF, n = 14 for BDNF and anti-SV30, and n = 5 for anti-SV30. *P < 0.05 BDNF versus aCSF, **P < 0.01 BDNF versus aCSF, #P < 0.05 BDNF versus BDNF + anti-SV30, ##P < 0.01 BDNF versus BDNF + anti-SV30, §P < 0.05 BDNF versus anti-SV30 and §§P < 0.01 BDNF versus anti-SV30.

preferentially to carbohydrate as the energy substrate. However, BDNF has been reported to increase energy expenditure (7, 11). This apparent discrepancy could be a result of the different methods of analysis; VO_2 is whether normalised with total body weight or not. Because it has recently been shown that normalising VO_2 with total body weight would overestimate the energy expenditure (27), we analysed energy expenditure based on the VO_2 and VCO_2 data unnormalised. Taken together, it is likely that BDNF reduces body weight primarily by suppressing energy intake.

It was reported that intra-PVN injection of urocortin decreases the RQ and increases body temperature (18, 28), in compatible with our result. Thus, it is suggested that urocortin mediates BDNF action to regulate lipid metabolism and body temperature.

Single i.c.v injection and continuous i.c.v infusion of BDNF was reported to increase plasma corticosterone (15, 16). By contrast, local infusion of BDNF into the PVN did not change plasma corticosterone level in the present study. Although this discrepancy cannot be explained at present, i.c.v administrated BDNF may affect corticosterone level via a mechanism not involving the PVN, as discussed by Nert *et al.* (16).

The results obtained in the present study suggest that the BDNF-CRH/urocortin pathway is a potential therapeutic target for treating patients with obese hyperphagia. As one of critical steps for this, Cao *et al.* (29) recently developed a molecular autoregulatory system using specific microRNA, which provides overexpression of BDNF at an optimal level without causing cachexia.