Brain-derived neurotrophic factor in the hypothalamic paraventricular nucleus increases energy expenditure by elevating metabolic rate

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Wang C, Bomberg E, Billington C, Levine A, Kotz CM. Brain-derived neurotrophic factor in the hypothalamic paraventricular nucleus increases energy expenditure by elevating metabolic rate. Am J Physiol Regul Integr Comp Physiol 293: R992–R1002, 2007. First published June 13, 2007; doi:10.1152/ajpregu.00516.2006.—Brain-derived neurotrophic factor (BDNF) decreases food intake and body weight, but few central sites of action have been identified. The hypothalamic paraventricular nucleus (PVN) is important in energy metabolism regulation, and expresses both BDNF and its receptor. We tested three hypotheses: 1) PVN BDNF reduces feeding and increases energy expenditure (EE), 2) PVN BDNF-enhanced thermogenesis results from increased spontaneous physical activity (SPA) and resting metabolic rate (RMR), and 3) PVN BDNF thermogenic effects are mediated, in part, by uncoupling protein 1 (UCP1) in brown adipose tissue (BAT). BDNF (0.5 μg) was injected into the PVN of Sprague-Dawley rats; and oxygen consumption, carbon dioxide production, food intake, and SPA were measured for 24 h in an indirect calorimeter. SPA was also measured in open-field activity chambers for 48 h after BDNF injection. Animals were killed 6 or 24 h after BDNF injection, and BAT UCP1 gene expression was measured with quantitative real-time PCR. BDNF significantly decreased food intake and body weight gain 24 h after injection. Heat production and RMR were significantly elevated for 7 h immediately after BDNF injection. BDNF had no effect on SPA, but increased UCP1 gene expression in BAT at 6 h, but not 24 h after injection. In conclusion, PVN BDNF reduces body weight by decreasing food intake and increasing EE consequent to increased RMR, which may be, in part, to BAT UCP1 activity. These data suggest that the PVN is an important site of BDNF action to influence energy balance.

BDNF reversed the phenotype of these animals (26), indicating that BDNF in the central nervous system exerts negative effects on feeding and body weight. Only a few studies have reported on BDNF energy expenditure (EE) effects. Nakagawa et al. (40) reported that subcutaneous injection of BDNF for 3 wk increased body temperature and oxygen (O2) consumption in db/db mice compared with that in paired animals. Intracerebroventricular administration of BDNF significantly reversed cold-induced reductions in body temperature in db/db mice (54). BDNF also enhanced norepinephrine turnover and increased levels of uncoupling protein 1 (UCP1; an indicator of thermogenesis) mRNA and protein in brown adipose tissue (54), suggesting positive regulation of thermogenesis and EE by BDNF.

The PVN is highly important to the regulation of energy metabolism. Several neuropeptides in the PVN affect energy metabolism, and some also influence physical activity. Neuropeptide Y (NPY; 7, 14) stimulates feeding, decreases EE, and does not affect physical activity (55). Agouti-related peptide, the agonist of the melanocortin 4 receptor, stimulates eating (19, 58) and reduces EE concomitant with reduced locomotion (52). Conversely, increased leptin expression in the PVN significantly reduces energy intake and enhances EE (4). Both urocortin (15, 16, 30) and corticotropin releasing factor (CRH) (35, 36) in the PVN suppress feeding and increase sympathetic activity, O2 consumption, and UCP gene expression. CRH at low doses increases and at high doses decreases locomotion (38). Orexin A in the PVN increases SPA (28) and EE (3, 28, 56).

Studies of specific brain areas important to BDNF effects on EE and SPA have not been reported. In the present study, we injected BDNF into the PVN and measured EE by indirect calorimetry and SPA with an activity monitoring system. We tested three hypotheses: 1) injection of BDNF into the PVN will reduce food intake and increase EE, resulting in a decrease in body weight gain; 2) changes in EE induced by BDNF result from heat produced by increased SPA and resting metabolic rate (RMR); 3) PVN BDNF thermogenic effects are mediated in part by UCP1 in brown adipose tissue. Our findings indicate that a single injection of BDNF in the PVN reduces feeding and body weight gain and increases EE by elevating resting metabolic heat production, but does not change SPA levels. We also found that BDNF in the PVN increases UCP1 expression in brown adipose tissue in the first few hours postadministration, which may contribute to enhanced RMR and EE.

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METHODS

Animals

Male Sprague-Dawley rats (Harlan, Madison, WI) weighing 280–320 g were housed individually in cages with a 12:12-h light-dark photo cycle (lights on at 07:00) in a room at 21–22°C. Teklad lab Chow and water were allowed ad libitum, except where noted. The protocol was approved by the Veterans Affairs Medical Center Institutional Animal Care and Use Committee.

Cannulation and Verification of Placement

Rats were anesthetized with intramuscular xylazine (3.5 mg/kg) and ketamine (20 mg/kg) and were fitted with a 28-gauge stainless steel guide cannula placed just above the PVN bilaterally. Stereotaxic coordinates were determined from the rat brain atlas of Paxinos and Watson (45) and are as follows: 0.6 mm lateral and 1.9 mm posterior to bregma, and 7.1 mm below the skull surface. The injector extended 1 mm further than the end of the guide cannula. The animals were given at least 1 wk to recover following surgery before experimental trials. After terminal experiments, the rats were decapitated and whole brain tissues were taken out and soaked in 10% formalin solution for at least 48 h. The brain tissues were sectioned with cryostat at thickness of 50 μm, mounted on gelatin-coated slides, stained with 0.1% thionin, and treated with ethanol (from 30% to 100%) and Clearing Agent (Electron Microscopy Sciences, Hatfield, PA). After the slides were dried, injection placement was determined microscopically at ×10 magnification, using the brain atlas of Paxinos and Watson (45) as a reference. Injection placements for experiments 1 and 2 are summarized in Fig. 1. A cannula was deemed correct if the histological examination indicated that the injection was within a 0.25-mm diameter from the targeted site. This rationale is based on diffusion coefficients of injection volume delivered (41). Data from animals with misplaced cannulae were excluded from the data analysis. Histological examination of brain tissue to verify injection site was not possible in the UCP1 gene expression experiment, because the brain tissue was used for another assay. Thus for animals participating in the present study we used feeding response to injected NPY as a behavioral assay to determine correct cannula placements. We deemed that injection sites were correctly targeted when a rat had a 2.0-gram increase in food intake within 2 h after injecting 100 pmol of NPY. This biological/behavioral assay has been verified in our previous work (7). Although this method errs on the side of overestimating incorrect cannula placements, it increases the probability of correct cannula placements in the remaining rats.

Drugs

BDNF was kindly provided by Regeneron Pharmaceuticals (Tarrytown, NY) and stored at −70°C at 10 mg/ml of 150 mM NaCl, 10 mM NaHPO₃ buffer, and 0.004% Tween 20 until use. BDNF was diluted with artificial cerebrospinal fluid (aCSF) just before use.

Injections

A volume of 0.5 μl was injected slowly over 30 s, with the injector left in place an additional 10 s to ensure extrusion from the tip, and to minimize distribution of drug upward on the cannula tract. Injections were limited as much as possible, and injection sides were utilized as evenly as possible; however, in some cases, a treatment day had to be repeated (due to issues, such as loss of building power, which affected the operation of the calorimetry and SPA chambers) and thus the

Fig. 1. Location of placed injections in the paraventricular nucleus of hypothalamus (PVN). A: placement from 7 rats in the experiment with indirect calorimetry. B: placement from 15 rats in the experiment of spontaneous physical activity (SPA) in open-field activity chambers. Two rats without correct placement were excluded from the statistical analysis. [From Paxinos and Watson (45).]
range of possible injection numbers is reported. In total, animals received less than or equal to 10 injections on one side, including injections of BDNF, aCSF, NPY (for another experiment), or k252a (TrkB antagonist; for another experiment). Injection sites were examined by light microscopy for tissue damage in the present studies and none was found. We also injected a dye (pontamine blue) in the PVN to determine potential diffusion and whether this was affected by repeated injections. Five rats were injected only once with 0.5 μl of 0.5% pontamine blue dye, and another five rats were injected with aCSF once per day for 9 days. For the last injection, the rats were injected with 0.5% pontamine blue dye. Brain tissue was taken out, stored at −20°C, and sectioned by cryostat at 20 μm. The sections were mounted on gel-coated slides. Fig. 2 shows the injection sites with one injection (Fig. 2, A and B) and 10 injections (Fig. 2, C and D). The diffusion radius was negligible for rats that underwent just one injection, and <0.25 mm for animals that underwent 10 injections, indicating limited diffusion from the injection site. This is in agreement with theoretical calculations of diffusion that incorporates ligand uptake at the injection site (41). However, dye presence is more suited as a marker for placement and not as an indication of injectate spread, as dye spread “is unlikely to recapitulate the extent of the spread of the injected peptide.” (27).

Specific Experimental Designs

Experiment 1. Food intake, EE, and SPA in an indirect calorimeter chamber. This experiment was designed to measure food intake, EE, and SPA simultaneously. Twenty-four-hour EE was measured using a customized, high-precision, single-chamber indirect calorimeter (Columbus Instruments, Columbus, OH). Before each single experiment, the calorimeter was calibrated by using a primary gas standard, and the rat was placed inside the test chamber with food and water. The chamber was sealed, and room air was pumped through the chamber at 3–4.7 l/min depending on animal body weight.

During EE measures, levels of SPA were also recorded using Opto-M Verimex Minor activity monitors (Columbus Instruments) placed around the indirect calorimeter. This device contains 45 col-liminated infrared activity sensors that detect horizontal and vertical movement, as well as ambulatory movements, which exclude repetitive signals from a single beam.

This system allows us to measure continuous metabolic indices (O2 consumption and CO2 production) and physical activity at a user-defined interval, in this case every 10 s. As such, we are able to identify periods when the animals had elevated metabolism due to physical activity and thereby quantify not only resting metabolism but also the EE of SPA.

Each rat was allowed to acclimate in a 14-liter, 30-cm-diameter, 20-cm-high cylindrical chamber in the testing area for up to 24 h prior to testing, during which time most animals maintained or had a small increase in their body weights. Between 10:00 AM and 12:00 PM the next morning, the animals were injected with 0.5 μg BDNF or aCSF and then stayed in the chamber for an additional 24 h. The dose of 0.5 μg BDNF was effective in reducing feeding and body weight without inducing a conditioned taste aversion (see companion manuscript, Ref. 55a). Using a counterbalanced design, each animal received both treatments with at least 48 h between treatments. The timing of the gas measurements was as follows: reference air was allowed to settle for 120 s, and then measured for 60 s. This was then followed by a period in which cage air was allowed to settle for 120 s and then measured for 10 s in each epoch for 120 epochs. This measurement protocol was repeated every 20 min.

The experimental measurements included food intake, body weight change, SPA, O2 consumption, CO2 production, and heat production. O2 consumption and heat production were normalized by mean individual body weight, using the average of body weights at the beginning and end of the experiment. Individual activity counts for horizontal, vertical (standing and rearing), and ambulatory movement were collected, and total SPA counts (the sum of all beam break counts) were calculated.

If there were zero SPA counts during a 10-s epoch, the status in this period was defined as resting; otherwise, the status was defined as SPA. EE during resting (tEE) within each hour and 24-h period and the duration of resting (in minutes) in each hour and 24-h period were

Fig. 2. Representative photographs of histology of the hypothalamic PVN following dye injections into the PVN. A: PVN histology (under microscope at ×4 magnification) after 1 injection of 0.5 μl of 0.5% pontamine blue dye. B: PVN histology under microscope at ×10 magnification from the boxed area in A. C: PVN histology (under microscope at ×4 magnification) after 10 injections of 0.5 μl aCSF once per day for 9 days. For the last injection, the rats were injected with 0.5% pontamine blue dye. D: PVN histology under microscope at ×10 magnification from the boxed area in C. Brain tissue was taken out, stored in −20°C, and sectioned by cryostat at 20 μm. Sections were mounted on gel-coated slides without staining. Black bar = 0.1-mm distance. The dark blue beyond the injection tip in B and D is pontamine blue dye particle. 3v, Third ventricle; F, fornix; P, PVN.
24-h mean RMR (mRMR) was defined as 24-h total rEE divided by calculated. Hourly average RMR (hRMR) was calculated by dividing hourly rEE by the resting time (in minutes) in the same period. The 24-h mean RMR (mRMR) was defined as 24-h total rEE divided by total resting time (in minutes). All above metabolic measurements and calculations were normalized for body weight, and are thus expressed as kcal/kg for total EE and rEE, and kcal·min⁻¹·kg⁻¹ for hRMR and mRMR. In this experiment, correct cannula placement of seven rats was verified by histology as shown in Fig. 1A, and five rats by response to PVN NPY administration as indicated above.

Experiment 2. SPA in open-field chamber activity monitors. In a separate group of animals, SPA was monitored in 43.2 cm

Experiment 3. The effect of BDNF on gene expression of UCP1 in brown adipose tissue. In this experiment, UCP1 gene expression was measured at 6 h and 24 h after administration of 0.5 μg BDNF. For the 6-h measurement, 19 rats (depicted NPY-responsive) were injected with aCSF (n = 9) or 0.5 μg BDNF (n = 10), and food was removed after injection. The animals were killed 6 h after injection by rapid decapitation. Another set of NPY-responsive rats (n = 27), were divided into three groups with body weight distributed evenly: 1) aCSF injected, 2) BDNF injected, and 3) aCSF injected with food intake yoked to that of the BDNF-treated rats (paired). The nine rats that were to receive BDNF (0.5 μg) and five rats that were to receive control (aCSF) injections were injected on the first day; the nine rats to receive aCSF and be paired and four additional controls (aCSF) were injected on the second day at 10:30 AM. Food was given at 4:30 PM ad libitum, with the exception of the paired group who were given the same amount of food that the BDNF-treated animals had consumed the previous day. At 8:00 AM the next morning, food was removed, and overnight food intake was measured. These animals were killed at 10:30 AM by rapid decapitation.

Interscapular and perirenal brown adipose tissues were taken for measurement of UCP1 mRNA. Total RNA from all tissues was extracted by the rapid guanidine thiocyanate-phenol-chloroform method (12). Real-time RT-PCR was used to measure relative UCP1 gene expression.

**REAL-TIME, ONE-STEP RT-PCR** The primers for UCP1 and the housekeeping gene ribosomal protein L32 (RPL32), were created using MacVector 7.2 (Accelrys, San Diego, CA; Table 1). One-step real-time RT-PCR was performed using 100 ng of total RNA and the reagents provided in the Roche RNA Amplification Kit SYBR Green I and a Roche LightCycler (Roche Applied Science, Indianapolis, IN). Real-time RT-PCR was performed as follows: reverse transcription for 30 min at 42°C, denaturation for 30 s at 95°C, followed by 35 cycles of cDNA amplification consisting of a 15-s denaturation at 95°C, primer annealing for 20 s at 61°C (UCP1) and 59°C (RPL32), and product elongation for 20 s at 72°C. Each primer set yielded a single product that corresponds to the appropriate nucleotide lengths. Data acquisition was taken at the end of each amplification cycle at a temperature slightly lower than the temperature required to melt the PCR product. Amplification products from PCR were purified (QIAquick PCR Purification Kit, Valencia, CA), determined by electrophoresis in a 4% Nusieve g gel, and then verified by capillary electrophoresis. The 2⁻ΔΔCT method was used to calculate relative UCP1 and RPL32 mRNA, and fold changes in mRNA levels (34). Fold change in UCP1 mRNA compared with RPL32 mRNA was expressed as the ratio of the mean relative UCP1 mRNA and mean relative RPL32 mRNA.

**Statistics**

Data were analyzed using StatView 5.0 (StatView, Cary, NC) and are expressed as means ± SE. For EE measurement, we first calculated individual hourly, 24-h total EE and EE for resting for each rat and analyzed these data by repeated-measures ANOVA to determine the effect of BDNF treatment on these end points. Repeated-measures ANOVA was also used to analyze food intake, body weight change, O₂ consumption, percent total EE for resting, RMR, duration of resting, and SPA counts. For UCP1 expression comparison, a Student’s t-test was used. A regression analysis was used to determine the association between total EE and total activity.

**RESULTS**

**Experiment 1. Food Intake, EE, and SPA in an Indirect Calorimeter Chamber**

Food intake and body weight. BDNF in the PVN significantly decreased feeding by 32.4% compared with rats treated with aCSF (19.2 ± 2.3 g for BDNF-treated rats vs. 28.5 ± 1.3 g for aCSF-treated rats, P = 0.0028) and significantly decreased body weight gain (−17.6 ± 4.3 g for BDNF-treated rats vs. −0.1 ± 1.1 g for aCSF-treated rats, P = 0.0033, Fig. 3).

O₂ consumption. BDNF significantly increased O₂ consumption by 8.9% (27,757.75 ± 668.21 ml/kg for BDNF-

![Fig. 3. Effect of brain-derived neurotrophic factor (BDNF) in the PVN on food intake (A) and body weight gain (B) during 24-h postinjection period. aCSF, artificial cerebrospinal fluid. #P < 0.01; n = 12.](image)
treated rats vs. 25,495.38 ± 498.36 ml/kg for aCSF-treated rats, \( P = 0.0079, \) Fig. 4).

**Total EE.** BDNF significantly increased 24-h EE by 9.74% (136.30 ± 3.37 kcal/kg for BDNF-treated rats vs. 124.20 ± 3.12 kcal/kg for aCSF-treated rats, \( P = 0.0013, \) Fig. 5, inset). During the 24-h period, the most dramatic increases in EE occurred within the first 7 h after BDNF injection (Fig. 5), although this pattern of increase was also observed in the late dark (17:20-h) and early light (21:24-h) cycle, with significant increases at the 15- and 23-h time points. Hourly EE in the BDNF-treated rats dropped significantly below that of the aCSF-treated rats at the 14-h time point only; at all other time points, EE in the BDNF-treated rats was either at the same level as or higher than that of the control rats (Fig. 5).

**SPA.** BDNF did not increase 24-h SPA as measured by beam breaks in the horizontal (42,326 ± 3,666 for BDNF-treated rats vs. 46,546 ± 2,842 for aCSF-treated rats, \( P = 0.2923 \)) and vertical (8,187 ± 1,059 for BDNF-treated rats vs. 9,916 ± 1,097 for aCSF-treated rats, \( P = 0.0891 \)) planes. Ambulatory activity counts were also not significantly different between groups (20,290 ± 2,727 for BDNF-treated rats vs. 22,781 ± 2,130 for aCSF-treated rats, \( P = 0.309 \)). Total SPA was calculated as the sum of the horizontal and vertical beam breaks. BDNF did not change total SPA counts in defined time intervals (0–6 h: 7,824 ± 720 for BDNF-treated rats vs. 7,052 ± 599 for aCSF-treated rats, \( P = 0.3734 \); 12-h dark cycle: 34,968 ± 4,288 for BDNF-treated rats vs. 37,740 ± 3,319 for aCSF-treated rats, \( P = 0.5073 \); 24-h after injection: 50,513 ± 4,640 for BDNF-treated rats vs. 56,461 ± 3,837 for aCSF-treated rats, \( P = 0.2231 \)). The time course analysis (Fig. 6) shows a similar pattern of SPA between BDNF and aCSF treatment for 13 h after administration. Then BDNF significantly decreased and increased SPA at 14 h (2,210 ± 500 for BDNF-treated rats vs. 4,020 ± 610 for aCSF-treated rats, \( P = 0.0017 \)) and 23 h (1,050 ± 180 for BDNF-treated rats vs. 461 ± 57 for aCSF-treated rats, \( P = 0.0111 \)) after injection, respectively.

To examine the relationship between total SPA and EE, a regression analysis was performed using data from control animals. As shown in Fig. 7, there is a significant correlation between total SPA and EE when animals were given aCSF (\( r = 0.716, P < 0.0001, \) Fig. 7A). When the same animals were treated with BDNF, this association no longer existed (\( r = 0.242, P = 0.255, \) Fig. 7B).

**Time spent resting and active.** There was no significant difference between groups in time spent resting (in minutes) in the 24-h period after injection (654.79 ± 20.95 min for BDNF-treated rats vs. 628.08 ± 11.34 min for aCSF-treated rats, \( P = 0.2207 \)) or ambulatory activity counts were also not significantly different between groups (20,290 ± 2,727 for BDNF-treated rats vs. 22,781 ± 2,130 for aCSF-treated rats, \( P = 0.309 \)). Total SPA was calculated as the sum of the horizontal and vertical beam breaks. BDNF did not change total SPA counts in defined time intervals (0–6 h: 7,824 ± 720 for BDNF-treated rats vs. 7,052 ± 599 for aCSF-treated rats, \( P = 0.3734 \); 12-h dark cycle: 34,968 ± 4,288 for BDNF-treated rats vs. 37,740 ± 3,319 for aCSF-treated rats, \( P = 0.5073 \); 24-h after injection: 50,513 ± 4,640 for BDNF-treated rats vs. 56,461 ± 3,837 for aCSF-treated rats, \( P = 0.2231 \)). The time course analysis (Fig. 6) shows a similar pattern of SPA between BDNF and aCSF treatment for 13 h after administration. Then BDNF significantly decreased and increased SPA at 14 h (2,210 ± 500 for BDNF-treated rats vs. 4,020 ± 610 for aCSF-treated rats, \( P = 0.0017 \)) and 23 h (1,050 ± 180 for BDNF-treated rats vs. 461 ± 57 for aCSF-treated rats, \( P = 0.0111 \)) after injection, respectively.
aCSF-treated rats, \( P = 0.0075 \), in the 12-h dark cycle by 12.18\% (28.96 ± 2.12 kcal/kg for BDNF-treated rats vs. 25.82 ± 1.17 kcal/kg for aCSF-treated rats, \( P = 0.0827 \)) and in the 0–24 h interval by 17.4\% (72.88 ± 3.52 kcal/kg for BDNF-treated rats vs. 62.07 ± 2.12 kcal/kg for aCSF-treated rats, \( P = 0.0019 \)) as shown in the inset of Fig. 8. The 24-h time course analysis (Fig. 8) indicates that BDNF significantly increased resting EE at 1, 2, 4, 7, 14, 17, 20, 21, and 22 h after injection. There was no difference in percent of total EE due to resting metabolism over the 24-h measurement period between aCSF- and BDNF-treated rats (53.49\% ± 2.22\% for BDNF-treated rats vs. 50.18\% ± 1.11\% for aCSF-treated rats, \( P = 0.1362 \)).

Mean RMR and hourly average RMR are shown in Fig. 9. BDNF significantly increased mean RMR by 12.12\% (0.111 ± 0.003 kcal/min/kg for BDNF-treated rats vs. 0.099 ± 0.002 kcal/min/kg for aCSF-treated rats, \( P = 0.0019 \)) as shown in the inset of the Fig. 9. The effect of BDNF on RMR occurred primarily in the first 7 h after injection; thereafter, there were no differences between groups except at hour 23 (Fig. 9).

EE during SPA. BDNF did not significantly increase EE during SPA (63.42 kcal/kg ± 3.44 for BDNF-treated rats vs. 62.13 ± 2.69 kcal/kg for aCSF-treated rats, \( P = 0.6535 \)).

Experiment 2. BDNF Effects on SPA in Open-Field Chamber Activity Monitors

There were no significant differences in time spent resting or time spent active during the 0- to 4-h, 4- to 24-h, 24- to 48-h, and 0- to 48-h periods (table 2). There were also no significant differences during the first 12-h dark cycle period (data not shown). These observations are consistent with the measurements from the Opto-M Verimex Minor activity monitors that were used in conjunction with the indirect calorimetry measurements.

Experiment 3. Effect of BDNF on Gene Expression of UCP1 in Brown Adipose Tissue

As shown in Fig. 10, 6 h after injection of 0.5 \( \mu \)g BDNF, UCP1 expression increased in interscapular brown adipose tissue by 53.3\% (1.522 ± 0.151 for BDNF-treated rats vs. 0.993 ± 0.128 for aCSF-treated rats, \( P = 0.0173 \)) and in perirenal brown adipose tissue by 42.2\% (1.641 ± 0.210 for
BDNF-treated rats vs. 1.154 ± 0.085 for aCSF-treated rats, 
$P = 0.0671$), respectively, as measured by RT-PCR (Fig. 10).
At 24 h after injection, although BDNF increases UCP1 ex-
pression in the interscapular brown adipose tissue by 37.5% and 36.9% compared with groups of aCSF and pairfed, re-
spectively (1.905 ± 0.297 for BDNF-treated rats, 1.385 ± 0.169 for aCSF-treated rats, and 1.391 ± 0.275 for pairfed rats, respectively), there was no significant difference (BDNF vs. aCSF; $P = 0.148$, and BDNF vs. pairfed: $P = 0.227$) as measured by RT-PCR (data not shown).

**DISCUSSION**

Our studies demonstrate that bilateral administration of 
BDNF in the PVN significantly reduces body weight. Loss of 
body weight in these animals is due to decrements in food 
intake and increases in EE. The enhanced EE in BDNF-treated 
animals results from elevated metabolic rate but not from 
changes in SPA. The present study is the first to demonstrate 
BDNF action in the PVN and to describe the components of EE 
affected by BDNF.

Feeding inhibition is one factor that contributed to the 
reduction in body weight gain observed in BDNF-treated 
animals. A one-time bilateral injection of BDNF in the PVN 
significantly reduced food intake by 32% (19.2 ± 2.3 g for 
BDNF-treated rats vs. 28.5 ± 1.3 g for aCSF-treated rats, Fig. 
3) and significantly decreased body weight gain (−17.6 ± 4.3 g for BDNF-treated rats vs. −0.1 ± 1.1 g for aCSF-treated rats) over a 24-h period. This observation is consistent with other reports of long-term BDNF administration. Ono et al. (44) reported that subcutaneous BDNF at 20 mg·kg$^{-1}$·day$^{-1}$ for 14 days significantly decreased feeding by $\sim$30% and body weight gain by $\sim$15% in female db/db mice. In a study of rats, BDNF at 0.375, 1.5, 3.0, and 6.0 g in the lateral ventricle for 
14 days, dose-dependently reduced feeding and body weight 
gain (46). Bariohay et al. (6) reported that infusion of BDNF 
(0.1 and 1 µg/day) into the dorsal vagal complex of rats for 14
days also decreased feeding and body weight gain, with feeding inhibition of 54.7% for the 0.1-µg dose and 67.4% for the 1-µg dose on the first measurement day and gradual recovery over time. The potency of inhibition between these studies and ours is different, likely due to factors including dose range, site of administration, experimental protocol, and species.

Increased EE also contributed to the weight loss observed after BDNF administration. BDNF in the PVN increased O$_2$ consumption by 8.9% and total EE by 9.74% during the 24-h postinjection period. Time course analysis indicates that BDNF increased EE most robustly in the first 7 h after injection (Fig. 5).

The above observations support the idea that BDNF decreases body weight gain both by reducing feeding and increasing EE. Several central mediators of energy balance have effects on EE that are related to changes in SPA. Orexin A in the PVN (28, 29, 31) and the rostral lateral hypothalamus increases SPA and EE (3). Agouti-related peptide in the PVN decreased SPA (52) and O$_2$ consumption (3). Ob/ob mice lacking both melanin-concentrating hormone and leptin had a decrease in body fat and an increase in EE resulting from both increased resting EE and locomotor activity (50). BDNF heterozygous mutant mice with low BDNF expression had higher locomotor activity compared with wild-type mice (26), suggesting that BDNF may reduce locomotor activity. To determine whether BDNF in the PVN affects SPA and the EE associated with such, continuous SPA was measured simultaneously with gas exchange measurements in indirect calorimeter chambers fashioned with activity monitoring devices and then separately measured in activity monitoring chambers. The measures taken in both chambers indicated that BDNF in the PVN did not significantly affect SPA (expressed as beam breaks) or in the time spent being active (Table 2). This suggests that BDNF in the PVN increases EE without affecting physical activity. Our results are inconsistent with a recent report (39) in which continuous lateral ventricle infusion of BDNF significantly increased locomotor activity; together, these data suggest that locomotor responses are based on site of administration.

The next goal was to determine whether BDNF increases RMR. We defined the rat’s status as resting if there were no SPA counts within a 10-s epoch, and reEE (kcal/kg) was determined by collecting EE data in these defined resting epochs. As shown in Fig. 8, BDNF significantly increased reEE during the 0- to 6-h and 0- to 24-h intervals. A time course analysis indicated that BDNF increased hourly reEE at 1, 2, 4, 7, 14, 17, 20, 21, and 22 h after injection. The similar patterns of SPA between the two treatments during 0–7 h (Fig. 6) and increased reEE by BDNF in the same period (Fig. 8) further suggest that BDNF increases energy metabolism without changes in SPA. The time course of reEE with BDNF treatment showed a biphasic pattern (Fig. 8): low in the dark phase and high in the light phase. This biphasic pattern can be explained by differences in time spent resting within each period as follows. Animals were injected ~3–5 h into the light phase. Measurements were made throughout the next 24 h, which thus included ~7–9 h of additional light phase, 12 h of dark phase, and then the first 3–5 h of the next light phase. During the first light phase, BDNF-treated rats spent more time resting and less time engaged in SPA, yet EE in BDNF-treated rats was increased during this time, and thus reEE was increased. During the dark phase, animals spent less time resting and more time engaged in physical activity compared with the light phase (Fig. 6), resulting in a low reEE during that period. During the second light phase, the BDNF-treated rats again spent more time resting and had low levels of SPA, resulting in increased reEE during the second light phase. The combination of high reEE during the first and second light phase and low reEE in the dark phase contributed to the biphasic pattern.

We then calculated hourly (hRMR; kcal·min$^{-1}$·kg$^{-1}$) and 24-h RMR (mRMR; kcal·min$^{-1}$·kg$^{-1}$) as shown in Fig. 9. BDNF significantly increased 24-h mRMR and hRMR during the first few hours after injection. Together these data suggest that BDNF increases EE primarily by increasing RMR. The timing of BDNF effects on reEE (Fig. 8) and hRMR (Fig. 9) are consistent with the effects on total EE (Fig. 5), further suggesting that increases in total EE are the result of increases in hRMR. These findings are similar to that reported for leptin.

**Table 2. Measurements with open-field chamber activity monitors after injection of aCSF and BDNF in the PVN**

<table>
<thead>
<tr>
<th>Time After Injection, h</th>
<th>Total SPA, counts</th>
<th>Distance Traveled, cm</th>
<th>Resting Time, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>aCSF</td>
<td>BDNF</td>
<td>aCSF</td>
<td>BDNF</td>
</tr>
<tr>
<td>0–4</td>
<td>9,547±703</td>
<td>9,256±604</td>
<td>8,046±664</td>
</tr>
<tr>
<td>4–24</td>
<td>49,368±3,647</td>
<td>47,463±3,448</td>
<td>37,172±3,631</td>
</tr>
<tr>
<td>24–48</td>
<td>64,386±3,877</td>
<td>63,744±3,064</td>
<td>52,047±5,253</td>
</tr>
<tr>
<td>0–48</td>
<td>12,330±5,752</td>
<td>12,046±6,076</td>
<td>97,265±5,808</td>
</tr>
</tbody>
</table>

The data are expressed as mean ± SE. aCSF, artificial cerebrospinal fluid; BDNF, brain-derived neurotropic factor; PVN, paraventricular nucleus; SPA, spontaneous physical activity. Total SPA is sum of ambulant, jumping, vertical, and steric counts.
which increases EE via increased RMR without changes in physical activity (18).

The primary efferent pathway regulating EE is believed to be the sympathetic nervous system, which heavily innervates thermogenic target tissues, such as brown adipose tissue. The PVN regulates outflow of the sympathetic nervous system and PVN stimulation induces sympathetic activity, including increases in heart rate and mean blood pressure, while PVN inhibition decreases sympathetic activity (1, 24, 25, 47). Recently, sympathetic innervation from PVN to intercapsular brown adipose tissue was reported (5, 10), and it is possible that PVN-injected BDNF may induce sympathetic activation of brown adipose tissue, leading to increased UCP1 activity and heat production. UCP1 is a mitochondrial protein expressed exclusively in brown adipose tissue (although it can be induced in white adipose tissue (22)) that mediates thermogenesis. UCP1 has a significant role in maintaining body temperature in rodents. To investigate whether BDNF increases thermogenic capacity, we measured UCP1 expression in brown adipose tissue 6 and 24 h after administration of BDNF to reflect measured time intervals in which BDNF stimulates (6-h, Fig. 9) and does not stimulate (24-h, Fig. 9) RMR. BDNF in the PVN increased UCP1 expression in both intercapsular and perirenal brown adipose tissue 6 h after administration (Fig. 10). We did not find a significant difference in UCP1 gene expression in either brown adipose tissue depot 24 h after BDNF administration, which is consistent with the lack of BDNF effect on RMR 24 h after administration (Fig. 9). These data are consistent with reports in which subcutaneously injected BDNF increased brown adipose tissue UCP1 expression 4 h postinjection (54) and chronic peripheral BDNF infusion increased UCP1 mRNA and protein and norepinephrine turnover in intercapsular brown adipose tissue (43). Our findings of BDNF-induced increases in EE, RMR, and UCP1 expression are also consistent with reports showing that BDNF-treated animals in a cold (54) or food-restrictive (40) situation maintained their body temperature, while controls did not. These findings suggest that BDNF may increase EE, in part, by stimulating UCP1 expression in brown adipose tissue.

One potential mechanism by which BDNF in the PVN affects feeding and EE is via CRH. Intracerebroventricular injection of BDNF increases CRH mRNA (20, 39) and decreases CRH peptide in the PVN (20), and CRH reduces food intake while increasing brown adipose tissue thermogenesis (2, 32), PVN CRH neurons project to the locus coeruleus (48) and rostral ventrolateral medulla (37), sites important to sympathetic activity, and intracerebroventricular CRH prolongs elevation of plasma epinephrine and norepinephrine and increases O2 consumption (8).

Another potential mechanism by which BDNF could affect sympathetic activity is by reducing GABAergic activity in the PVN. GABA reduces sympathetic outflow from the PVN (11, 17, 47, 63), and functional changes in GABA_A and GABA_B receptors may regulate sympathetic outflow (33). BDNF induces a rapid reduction in postsynaptic GABA_A receptor number, while k252a (TrkB antagonist) blocked this effect (9). BDNF reduces both evoked and spontaneous inhibitory postsynaptic currents within 5 min and inhibits GABA_A synaptic responses by postsynaptic activation of TrkB receptor (51). PVN BDNF also decreases frequency of miniature inhibitory postsynaptic currents in GABA postsynapses, increases endocytosis of GABA_A receptors, and decreases availability of postsynaptic GABA_A receptors (21).

As BDNF is a cytokine, it is possible that BDNF effects on energy metabolism are related to a role in the inflammation process, particularly as other cytokines have been shown to affect appetite and energy metabolism (23, 53, 59). We have shown that BDNF injections do not result in illness or malaise (results from conditioned taste aversion experiment in the companion paper, Ref. 55a), and the idea that potential inflammation effects of BDNF are responsible for the energy metabolism effects observed in the present study is inconsistent with the finding that elevated cytokines are usually associated with obesity, whereas low cytokine levels are associated with weight loss (42). In the current studies of enhanced BDNF levels by exogenous BDNF injection, we observed weight loss rather than weight gain. Furthermore, Kernie et al. (26) reported that among the mice heterozygous for targeted disruption of BDNF, NT4/5, NT3, TrkC, and TrkA, only BDNF mutants (low BDNF expression) showed significant weight gain, and only BDNF and NT4/5 (both agonists of TrkB) reversed the phenotype of the BDNF heterozygous mice. Also, in the BDNF mutants with obesity, BDNF expression was reduced, including decrements in the hypothalamic PVN. This again indicates that low BDNF levels are associated with obesity, not weight loss, and suggests the importance of BDNF in energy regulatory processes, as the PVN is a major site of energy balance regulation. Indeed, Xu et al. (60) reported that BDNF plays a role downstream of melanocortin signaling. Recently, a case of severe human obesity was found to present with a mutation in the BDNF receptor TrkB (62). Together these studies suggest that BDNF plays a physiological role in energy metabolism.

In conclusion, our studies demonstrate that BDNF in the PVN reduces body weight gain via inhibition of food intake and enhanced EE due to increased RMR. BDNF in the PVN increased UCP1 expression in brown adipose tissue, which may contribute to elevated EE. Our studies indicate that BDNF did not affect SPA or the heat production associated with such. Together these data suggest that the PVN may be an important site of BDNF action in the regulation of energy balance.

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GRANTS

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REFERENCES


