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A Role for the Endogenous Opioid β-Endorphin in Energy Homeostasis

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Proopiomelanocortin (POMC) neurons in the hypothalamus are direct targets of the adipostatic hormone leptin and contribute to energy homeostasis by integrating peripheral and central information. The melanocortin and β-endorphin neuropeptides are processed from POMC and putatively coreleased at axon terminals. Melanocortins have been shown by a combination of pharmacological and genetic methods to have inhibitory effects on appetite and body weight. In contrast, pharmacological studies have generally indicated that opioids stimulate food intake. Here we report that male mice engineered to selectively lack β-endorphin, but that retained normal melanocortin signaling, were hyperphagic and obese. Furthermore, β-endorphin mutant and wild-type mice had identical orexigenic responses to exogenous opioids and identical anorectic responses to the nonselective opioid antagonist naloxone, implicating an alternative endogenous opioid tone to β-endorphin that physiologically stimulates feeding. These genetic data indicate that β-endorphin is required for normal regulation of feeding, but, in contrast to earlier reports suggesting opposing actions of β-endorphin and melanocortins on appetite, our results suggest a more complementary interaction between the endogenously released POMC-derived peptides in the regulation of energy homeostasis. (*Endocrinology* 144: 1753–1760, 2003)

PROOPIOMELANOCORTIN (POMC) neurons in the hypothalamic arcuate nucleus (Arc) contribute to energy homeostasis by integrating peripheral and central information related to caloric balance and metabolism (1–5). These neurons are direct targets of the adipostatic hormone leptin, receive afferent signals from the medullary dorsal vagal complex, and project to other hypothalamic and brainstem nuclei that control feeding and autonomic responses (1, 2, 4, 5). POMC is processed stoichiometrically to melanocortins and β-endorphin, and both classes of neuropeptides are putatively coreleased at axon terminals (6). The role of melanocortins in the regulation of appetite and metabolism has been recently defined by pharmacological and genetic methods (7–11); however, the actions of endogenously released β-endorphin in energy homeostasis have not been characterized.

Pharmacological studies have generally indicated that opioids stimulate food intake (12, 13), opposite to the anorectic effect of melanocortins. However, it is impossible to mimic the actions of individual endogenous opioid pathways by pharmacological manipulation. β-Endorphin has a relatively high affinity for the μ, δ, and κ subtypes of opioid receptors (14), and exogenously applied β-endorphin can act at sites where it is not normally released, but that are the targets of other opioid peptide signaling pathways. Conversely, application of subtype-selective opioid receptor antagonists will interfere with multiple endogenous peptide signaling pathways. For these reasons we chose to differentiate the effects of β-endorphin on food intake from other endogenous opioid peptides, such as enkephalin and dynorphin, by genetic removal of β-endorphin.

Abbreviations: Arc, Arcuate nucleus; CNS, central nervous system; DEXA, dual energy x-ray absorptiometry; icv, intracerebroventricular; NPY, neuropeptide Y; POMC, proopiomelanocortin; Tg, transgenic.

Materials and Methods

Mice

We generated β-*END*$^{-/-}$ and β-*END*$^{+/+}$ mice either from heterozygous β-*END*$^{+/-}$ mating pairs or from homozygous β-*END*$^{-/-}$ or β-*END*$^{+/+}$ mating pairs. Mice were genotyped by PCR as described previously (15) and were N10 congenic to the C57BL/6J strain (The Jackson Laboratory, Bar Harbor, ME). The transgenic (Tg) rescue experiments were carried out in N5 congenic C57BL/6J animals; *Tg2* was previously termed *phal**, and *Tg13* was previously termed *pomc*27* (16, 17). The compound *Mc4r*$^{-/-}$/β-*END*$^{-/-}$ mice were generated by mating *Mc4r*$^{-/-}$ males (8) (N3 C57BL/6J) and β-*END*$^{+/-}$ (N10 C57BL/6J) females. Mice were housed under constant temperature in murine specific pathogen-free animal facilities with *ad libitum* access to water and rodent chow pellets (5% fat, 19% protein, and 5% fiber by weight; 3.4 g/kcal).

Experimental animals

All mice were housed in animal rooms dedicated to use by the Tg facility and located within the Department of Comparative Medicine. All procedures conformed to USPHS guidelines and were approved by the institutional animal care and use committee.

Growth curves, carcass analysis and dual energy x-ray absorptiometry (DEXA) scan

Mice were weighed weekly from weaning at 3 wk of age. At age 5 months mice were euthanized, and liver, spleen, kidney, heart, testis, and all fat pads were removed and weighed. A portion of the inguinal fat pads was placed in 4% paraformaldehyde, then dehydrated, embedded in paraffin. Fifteen-micrometer-thick sections were stained with hematoxylin and eosin. The percent body fat was determined by analyzing the carcass composition of mice by DEXA (PIXImus mouse densitometer, Lunar Corp., Madison, WI).

Food intake measurements

Mice were housed individually at 5 wk of age. They had *ad libitum* access to water and food pellets (5% fat, 19% protein, and 5% fiber; 3.4 g/kcal) provided in wire cage tops. Food was weighed daily, and average daily intake was calculated from consecutive measurements over 7 d after a 10- to 14-d acclimatization period to individual housing.

Oxygen consumption measurements

Oxygen consumption was determined for multiple animals simultaneously by indirect calorimetry using an Oxymax system (Columbus Instruments, Columbus, OH) as previously described (10).

Plasma insulin, glucose, leptin, and T_4 measurements

Mice were killed after a 16- to 20-h fast (fasted) or after *ad libitum* access to food (fed), and trunk blood was collected. T_4, insulin, and leptin levels were determined by RIA (Linco Research, Inc., St. Charles, MO) from sera. Glucose levels were measured from blood using a Basic One Touch glucometer (Lifescan, Johnson & Johnson, Milpitas, CA). All blood samples were obtained in the fed state from 5-month-old mice unless otherwise stated.

Glucose tolerance test

Mice were fasted for 16 h and then given an ip injection of 2 g/kg glucose. Glucose levels were determined from tail bleeds at 0, 15, 30, 45, 60, 90, 120, 150, and 180 min.

Insulin sensitivity test

Mice were fasted for 16 h and then given an ip injection of 0.75 U/kg insulin. Glucose levels were determined from tail bleeds at 0, 30, 60, and 90 min.

Immunocytochemistry

Mice were perfused with 4% paraformaldehyde, and hypothalamic (24-μm thick) and pituitary (12-μm thick) sections were prepared for immunostaining as described previously. A rabbit polyclonal antibody to β-endorphin (18) was diluted 1:5000 in 1 mg/ml BSA (fraction V) and 0.3% Triton X-100 in PBS for incubation with free-floating hypothalamic sections. The β-endorphin antibody was used in the same buffer at 1:1000 for staining slide-mounted pituitary sections. After washing the sections and incubating with biotinylated goat antirabbit IgG (Vector Laboratories, Inc., Burlingame, CA), the reaction product was visualized with an ABC Elite kit (Vector Laboratories, Inc.) and diaminobenzidine, followed by a methyl green counterstain.

β-Endorphin RIA

β-Endorphin levels were measured in both the hypothalamus and pituitary using a rat β-endorphin RIA kit (Phoenix Pharmaceuticals, Inc., Belmont, CA). The tissue was prepared as described in the manufacturer's instructions.

Intracerebroventricular (icv) injections

A 27-gauge stainless steel cannula was surgically placed into a lateral ventricle of anesthetized mice as described previously (9). The mice were allowed to recover for at least 1 wk. Neuropeptide Y (NPY; 1.5 μg; Peninsula Laboratories, Inc., Belmont, CA), 0.5 μg β-END (gift from Dr. A. Parlow), or saline in a 1.5-μl volume was injected through the cannula. Naloxone (10 mg/kg) or 0.9% saline vehicle was given ip 15 min before the icv injection. Food intake was measured each hour for 3 h after the injection. This short time period within the light phase of the animals' diurnal cycle was inadequate to reveal the basal difference between genotypes that was primarily evident during nocturnal feeding bouts.

Naloxone effects on food intake

Mice were fasted for 24 h and then given an ip injection of 10 mg/kg naloxone or 0.9% saline. After 15 min, food was returned, and food intake was measured for 1 h.

Statistics

Statistical analyses were performed using PRISM (GraphPad Software, Inc., San Diego, CA) or StatView (SAS Institute, Inc., Cary, NC). Growth curves, insulin sensitivity, and glucose tolerance tests were analyzed using a two-factor ANOVA with time as the repeated measure. All other data were analyzed using *t* test or ANOVA, followed by Bonferroni/Dunn *post hoc* analysis for paired group comparisons.

Results

Male mice lacking β-endorphin (β-$END^{-/-}$) are significantly heavier and have greater adiposity than wild-type (β-$END^{+/+}$) male mice

To examine the effect of β-endorphin deficiency on weight homeostasis we weighed both β-$END^{-/-}$ and β-$END^{+/+}$ mice over time. These growth curves demonstrated that male β-$END^{-/-}$ mice weighed significantly more than male β-$END^{+/+}$ mice, starting at 4 wk of age and continuing into adulthood [two-way repeated measures ANOVA: significant main effect of genotype ($P < 0.001$), time ($P < 0.0001$), and genotype × time interaction ($P < 0.0001$); df of genotype = 1, time = 9, genotype × time = 9; F values: genotype = 16.6, time = 1213, genotype × time = 4.5; n = 15–20; Fig. 1A]. In contrast, the weights of female β-$END^{-/-}$ mice differed only transiently from those of female β-$END^{+/+}$ mice between 4–8 wk of age ($P < 0.05$, by *t* test at each time point; n = 10–11; Fig. 1A). Body length and the weights of various organs, such as liver, spleen, kidney, heart, and testis, were not changed in either sex of β-$END^{-/-}$ mice (data not shown). However, both the inguinal/gonadal and retroperitoneal/perirenal white fat stores of male β-$END^{-/-}$ mice were 2-fold heavier than those of male β-$END^{+/+}$ mice ($P < 0.01$, by *t* test; n = 8), whereas intrascapular brown fat was not altered (Fig. 1B). Furthermore, male β-$END^{-/-}$ mice had 50% greater total body fat as measured by DEXA scan ($P < 0.05$, by *t* test; n = 8; Fig. 1C), and histological examination suggested hypertrophy of the adipocytes (Fig. 1D). The fat stores of female β-$END^{-/-}$ mice were not increased (data not shown). An identical sexually dimorphic phenotype with equivalent or greater male pattern obesity was observed in β-$END^{-/-}$ mice crossed onto either a 129S6/SV (6 months old: β-$END^{+/+}$ 33.9 ± 2.1 g, n = 8; β-$END^{-/-}$, 49.6 ± 1.9 g, n = 13) or outbred Swiss albino background. These data indicate that the development of obesity in β-endorphin-deficient mice is independent of the known genetic predisposition of C57BL/6 mice to gain excessive weight and adipose mass.

Male β-$END^{-/-}$ mice have increased food intake, but no overt change in basal metabolic rate

To determine the underlying mechanism for the increased weight and adiposity of the β-$END^{-/-}$ mice, we examined both their food intake and their basal metabolic rate. The average daily food intake of 7- to 8-wk-old male β-$END^{-/-}$ mice was significantly increased compared with that of β-$END^{+/+}$ males ($P < 0.05$, by *t* test; n = 8; Fig. 1E). To determine whether the increased feeding was due to atten-

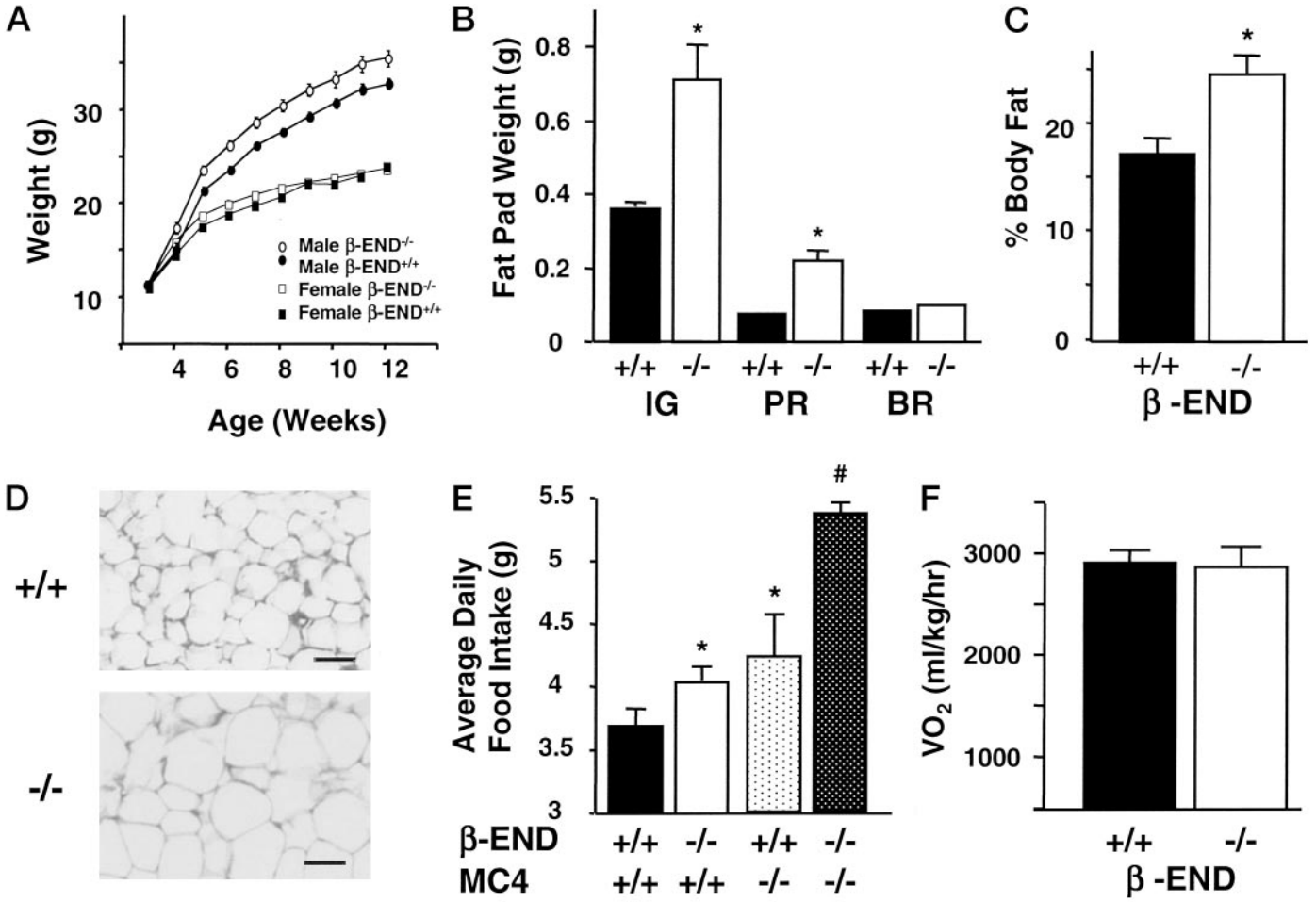

FIG. 1. Weight, fat mass, food intake, and oxygen consumption of β-*END*$^{-/-}$ and β-*END*$^{+/+}$ mice. A, Growth curves of sibling male and female β-*END*$^{-/-}$ and β-*END*$^{+/+}$ mice reared by β-*END*$^{+/-}$ parents (n = 11–24) (male mice, $P < 0.001$ for genotype, by repeated measure ANOVA). B, Weights of inguinal/gonadal (IG), retroperitoneal/perirenal (PR), and intrascapular brown (BR) fat pads in male β-*END*$^{-/-}$ and β-*END*$^{+/+}$ mice (n = 8; *, $P < 0.01$, by *t* test). C, Body fat percentage from DEXA scan analysis of male β-*END*$^{-/-}$ and β-*END*$^{+/+}$ mice (n = 8; *, $P < 0.05$, by *t* test). D, Morphology of inguinal fat from male β-*END*$^{-/-}$ and β-*END*$^{+/+}$ mice. *Scale bar*, 50 μm. E, Average daily food intake of β-*END*$^{+/+}$/*Mc4r*$^{+/+}$, β-*END*$^{-/-}$/*Mc4r*$^{+/+}$, β-*END*$^{+/+}$/*Mc4r*$^{-/-}$, and β-*END*$^{-/-}$/*Mc4r*$^{-/-}$ male mice (n = 6–8; *, $P < 0.05$ compared with wild-type mice; #, $P < 0.05$ compared with all other groups, by *t* test). F, Oxygen consumption in β-*END*$^{-/-}$ and β-*END*$^{+/+}$ mice. All data are expressed as the mean ± SEM.

uated αMSH biosynthesis or signaling, we generated compound homozygote mice lacking both β-endorphin and the MC4 receptor, the primary receptor mediating the anorectic effects of αMSH. The average daily food intake of β-*END*$^{-/-}$/*Mc4r*$^{-/-}$ mice was significantly higher than that of either β-*END*$^{-/-}$/*Mc4r*$^{+/+}$ or β-*END*$^{+/+}$/*Mc4r*$^{-/-}$ mice ($P < 0.05$, by *t* test; n = 6–8; Fig. 1E). β-*END*$^{-/-}$/*Mc4r*$^{-/-}$ mice were also significantly heavier than β-*END*$^{+/+}$/*Mc4r*$^{-/-}$ mice between 4 and 9 wk of age [two way repeated measures ANOVA: significant main effect of genotype ($P < 0.05$) and time ($P < 0.005$); df of genotype = 1, time = 3; F values: genotype = 6.78, time = 109.4; n = 7]. The weights of the mice (grams) were: at 4 wk: β-*END*$^{+/+}$/*Mc4r*$^{-/-}$, 17.6 ± 1.3; β-*END*$^{-/-}$/*Mc4r*$^{-/-}$, 21.5 ± 0.7; 7 wk: β-*END*$^{+/+}$/*Mc4r*$^{-/-}$, 27.7 ± 1.1; β-*END*$^{-/-}$/*Mc4r*$^{-/-}$, 31.9 ± 0.9; and 9 wk: β-*END*$^{+/+}$/*Mc4r*$^{-/-}$, 36.5 ± 1.0; β-*END*$^{-/-}$/*Mc4r*$^{-/-}$, 39.0 ± 1.0. The difference in weights was not significant after 10 wk of age, probably due to the large metabolic defect seen in the *Mc4r*$^{-/-}$ mice that overshadowed the additive genotype effect on food intake.

In contrast to the augmented food intake, there were no significant differences between β-*END*$^{-/-}$ and β-*END*$^{+/+}$ mice in their basal metabolic rate, as measured by oxygen consumption or respiratory quotient (Fig. 1F), or serum T_4 levels (β-*END*$^{+/+}$, 3.9 ± 0.1 ng/ml, n = 8; β-*END*$^{-/-}$, 3.5 ± 0.4 ng/ml, n = 7). These data are consistent with our previous findings that the basal core temperature and activity levels of the β-*END*$^{-/-}$ and β-*END*$^{+/+}$ male mice were also not significantly different (15, 19).

β-END$^{-/-}$ *mice are hyperinsulinemic and hyperleptinemic and have altered glucose homeostasis*

Previous studies have suggested that β-endorphin plays a role in the regulation of insulin and glucose homeostasis (20, 21). We therefore examined insulin and glucose homeostasis in β-*END*$^{-/-}$ mice. We found that 5-month-old β-*END*$^{-/-}$ male mice had elevated basal insulin levels [Fig. 2A; one-way ANOVA F(3,17) = 7.17; $P < 0.05$ for β-*END*$^{+/+}$ fed mice

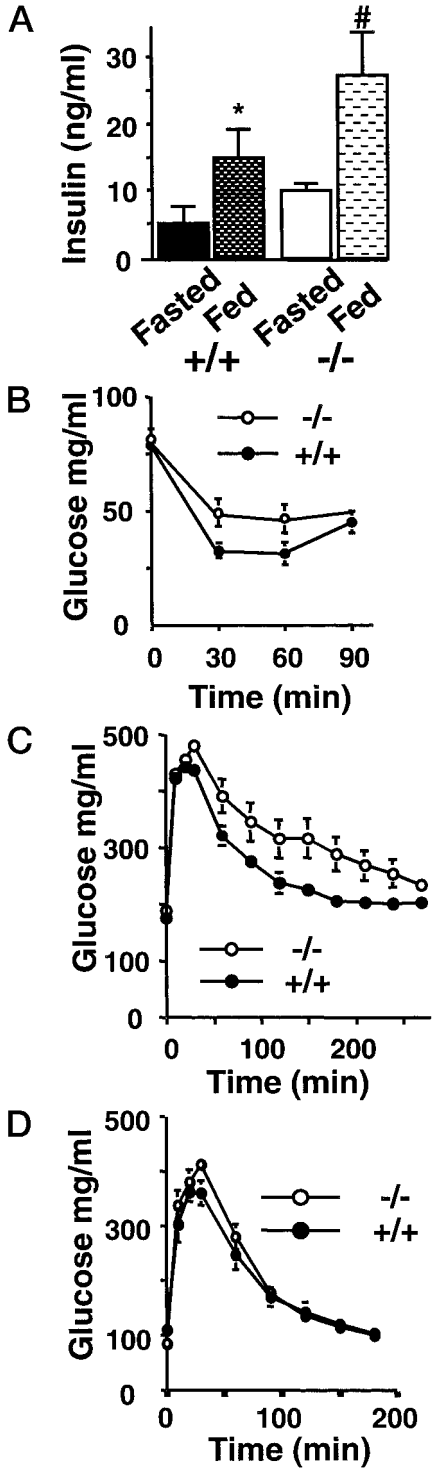

FIG. 2. Insulin and glucose homeostasis in β-*END*$^{-/-}$ and β-*END*$^{+/+}$ mice. A, Insulin levels in male β-*END*$^{-/-}$ and β-*END*$^{+/+}$ mice in the fed and fasted states (n = 5–6; *, $P < 0.05$ compared with β-*END*$^{+/+}$ fasted mice; #, $P < 0.05$ compared with β-*END*$^{+/+}$ fed mice and β-*END*$^{-/-}$ fasted mice, by one-way ANOVA). B, Insulin sensitivity test in male β-*END*$^{-/-}$ and β-*END*$^{+/+}$ mice (n = 8; $P < 0.01$ for genotype, by repeated measure ANOVA). C, Glucose tolerance test in male β-*END*$^{-/-}$ and β-*END*$^{+/+}$ mice (n = 6); $P < 0.005$ for genotype, by repeated measure ANOVA). D, Glucose tolerance test in female β-*END*$^{-/-}$ and β-*END*$^{+/+}$ mice. All data are expressed as the mean ± SEM.

compared with β-*END*$^{+/+}$ fasted mice; $P < 0.05$ for β-*END*$^{-/-}$ fed mice compared with β-*END*$^{+/+}$ fed mice and β-*END*$^{-/-}$ fasted mice) and exhibited an attenuated hypoglycemic response to insulin administration [Fig. 2B; two way repeated measures ANOVA: significant main effect of genotype ($P < 0.01$) and time ($P < 0.001$); df of genotype = 1, time = 2, genotype × time = 2; F values: genotype = 8.8; time = 5.1, genotype × time = 1.0; n = 8]. Furthermore, male β-*END*$^{-/-}$ mice were not able to clear glucose as efficiently as β-*END*$^{+/+}$ mice in a glucose tolerance test (Fig. 2C; two-way repeated measures ANOVA: significant main effect of genotype ($P < 0.005$), time ($P < 0.0001$), and genotype × time interaction ($P < 0.001$); df of genotype = 1, time = 12, genotype × time = 12; F values: genotype = 13.7, time = 141.0, and genotype × time F = 3.4; n = 6–7], although basal fasted and postprandial glucose levels were normal. In contrast, the nonobese female β-*END*$^{-/-}$ mice had normal glucose tolerance tests (Fig. 2D). However, nonobese male β-*END*$^{-/-}$ mice at 4 wk of age had normal glucose and insulin levels (weight: β-*END*$^{+/+}$, 19.4 ± 0.5 g, n = 6; β-*END*$^{-/-}$, 19.6 ± 0.4 g, n = 5; glucose: β-*END*$^{+/+}$, 143 ± 4.9 mg/ml, n = 6; β-*END*$^{-/-}$, 141 ± 11.8 mg/ml, n = 5; insulin: β-*END*$^{+/+}$, 0.7 ± 0.1 ng/ml, n = 6; β-*END*$^{-/-}$, 0.8 ± 0.1 ng/ml, n = 5). These results suggest that the modest hyperinsulinemia in adult males is probably secondary to the increased fat mass rather than due to a direct effect of the loss of β-endorphin. Examination of the levels of the adipostatic hormone leptin showed that adult male β-*END*$^{-/-}$ mice were also hyperleptinemic (β-*END*$^{+/+}$, 3.9 ± 0.7 ng/ml, n = 9; β-*END*$^{-/-}$, 8.3 ± 0.9 ng/ml, n = 10; $P < 0.01$, by *t* test) consistent with their increased adiposity.

Tissue-specific Tg rescue of β-endorphin in β-END$^{-/-}$ *mice*

To determine which site of β-endorphin release was responsible for the obesity phenotype in β-*END*$^{-/-}$ mice, we used a tissue-specific Tg rescue approach (Fig. 3A). *Tg2* contains 2 kb of the mouse *Pomc* promoter previously shown to be sufficient to express the gene in the pituitary, but not the central nervous system (CNS; Refs. 16 and 17). In contrast, *Tg13* has all the regulatory elements of the mouse *Pomc* gene required for Tg expression in the pituitary and CNS (3, 16). Both constructs have an in-frame oligonucleotide insertion in exon 3 that disrupts the biological activity of αMSH and ACTH (Fig. 3A). The two strains of Tg mice were crossed to β-*END*$^{-/-}$ mice, and progeny were examined by immunohistochemistry. *Tg2* rescued β-endorphin expression only in pituitary anterior lobe corticotrophs and intermediate lobe melanotrophs, whereas *Tg13* restored accurate cell-specific β-endorphin expression in both lobes of the pituitary and Arc neurons (Fig. 3B). The β-endorphin content in the pituitary (combined anterior and neurointermediate lobes) was restored to wild-type levels by both transgenes (β-*END*$^{+/+}$, 257 ± 19 ng/gland, n = 5; β-*END*$^{-/-}$, <0.16 ng/gland, n = 6; *Tg2*/β-*END*$^{-/-}$, 197 ± 8 ng/gland, n = 5; *Tg13*/β-*END*$^{-/-}$, 276 ± 42 ng/gland, n = 4). The levels of β-endorphin in β-*END*$^{+/+}$, *Tg2*/β-*END*$^{-/-}$, and *Tg13*/β-*END*$^{-/-}$ mice were not statistically different from each other. *Tg13* also restored quantitatively normal levels of hypothalamic β-endorphin in all rescued mice, whereas β-endorphin remained undetect-

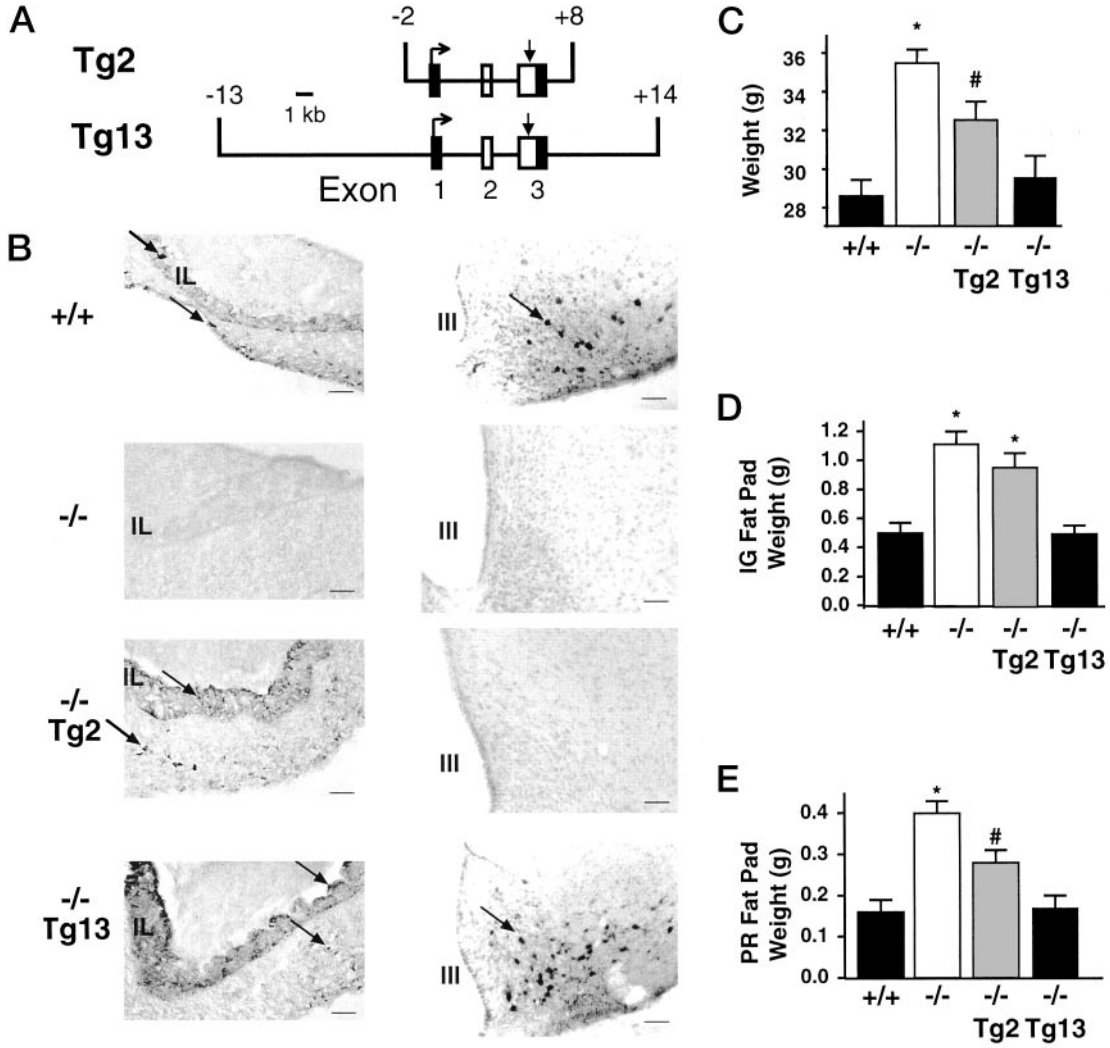

FIG. 3. Tg rescue of β-endorphin expression restores body and fat pad weights of 5-month-old male β-*END*$^{-/-}$ mice. A, Structure of *Pomc* transgenes. *Tg2* is a 10.2-kb genomic fragment that contains the entire transcriptional unit of the mouse *Pomc* gene and 2 kb of 5′- and 3′-flanking sequences. *Tg13* is a 27-kb genomic fragment that contains the entire transcriptional unit of the mouse *Pomc* gene and 13 kb of 5′- and 8 kb of 3′-flanking sequences. Both constructs have an in-frame 33-bp heterologous sequence (*arrow*) inserted into a blunted *Nco*I site in exon 3 that disrupts the coding sequence for ACTH and αMSH by the addition of 11 amino acids (CLSLSSLLSRM), but does not alter the processing or sequence of the carboxyl-terminal β-endorphin. The *boxes* indicate exon sequences and the *black*-shaded regions indicate untranslated sequences. B, Immunocytochemical localization of β-endorphin in the pituitary (*left*) and Arc (*right*) of β-*END*$^{+/+}$, β-*END*$^{-/-}$, and β-*END*$^{-/-}$ mice expressing the *Tg2* or *Tg13* transgene. The *arrows* indicate β-endorphin-immunoreactive pituitary corticotrophs and melanotrophs and hypothalamic neurons. IL, Intermediate lobe; III, third ventricle. *Scale bars*, 200 μm for pituitary sections; 50 μm for Arc sections. C, Body weights (n = 6–15; by one-way ANOVA: *, $P < 0.001$ compared with both β-*END*$^{+/+}$ and β-*END*$^{-/-}$/*Tg13*; # $P < 0.01$ compared with β-*END*$^{+/+}$, β-*END*$^{-/-}$, and β-*END*$^{-/-}$/*Tg13* mice). D, Weights of inguinal/gonadal (IG) fat pads (n = 6–15; by one-way ANOVA: *, $P < 0.01$ compared with both β-*END*$^{+/+}$ and β-*END*$^{-/-}$/*Tg13*). E, Weights of retroperitoneal/perirenal (PR) fat pads (n = 6–15; by one-way ANOVA: *, $P < 0.001$ compared with both β-*END*$^{+/+}$ and β-*END*$^{-/-}$/*Tg13*; #, $P < 0.05$ compared with β-*END*$^{+/+}$, β-*END*$^{-/-}$, and β-*END*$^{-/-}$/*Tg13*). All data are expressed as the mean ± SEM.

able in four of five hypothalami examined from the *Tg2* rescued mice, consistent with the immunohistochemical data (β-*END*$^{+/+}$, 484 ± 61 pg/hypothalamus, n = 5; β-*END*$^{-/-}$, <160 pg/hypothalamus, n = 7; *Tg2*/β-*END*$^{-/-}$, <160 pg/hypothalamus, n = 4; *Tg13*/β-*END*$^{-/-}$, 387 ± 27 ng/hypothalamus, n = 4). The one other *Tg2*/β-*END*$^{-/-}$ mouse had levels of immunoreactive β-endorphin just above RIA sensitivity, possibly due to trace contamination of the hypothalamic block by a fragment of the underlying pituitary intermediate lobe.

Tg rescue of β-endorphin to sites in the CNS is required for the full rescue of the obesity phenotype

Re-expression of β-endorphin to the pituitary by *Tg2* partially rescued both the body weight and fat pad weights of β-*END*$^{-/-}$ mice (Fig. 3, C–E). However, re-expression of β-endorphin to the pituitary and CNS by *Tg13* completely normalized body weight and fat mass of the β-*END*$^{-/-}$ mice (Fig. 3, C–E), suggesting that both central and pituitary β-endorphin expressions contribute to energy homeostasis [one way ANOVA: body weights (n = 6–15): $F(3,38) = 22.7$,

$P < 0.001$ for β-$END^{+/+}$ mice compared with β-$END^{-/-}$ mice, $P < 0.001$ for *Tg13*/β-$END^{-/-}$ compared with β-$END^{-/-}$ mice, $P < 0.01$ for *Tg2*/β-$END^{-/-}$ compared with β-$END^{-/-}$, β-$END^{+/+}$, and *Tg13*/β-$END^{-/-}$ mice; inguinal fat pad weights (n = 6–15): $F(3,38) = 11.6$, $P < 0.001$ for β-$END^{+/+}$ mice compared with β-$END^{-/-}$ mice, $P < 0.001$ for *Tg13*/β-$END^{-/-}$ compared with β-$END^{-/-}$ mice, $P < 0.01$ for *Tg2*/β-$END^{-/-}$ compared with β-$END^{+/+}$ and *Tg13*/β-$END^{-/-}$ mice; retroperitoneal/perirenal fat pad weights (n = 6–15): $F(3,38) = 11.9$, $P < 0.001$ for β-$END^{+/+}$ mice compared with β-$END^{-/-}$ mice, $P < 0.01$ for *Tg13*/β-$END^{-/-}$ compared with β-$END^{-/-}$, $P < 0.01$ for *Tg2*/β-$END^{-/-}$ mice compared with β-$END^{+/+}$ and β-$END^{-/-}$ mice].

NPY, β-endorphin, and naloxone effects on food intake in β-$END^{-/-}$ mice

We next examined whether the response of β-$END^{-/-}$ mice to food intake stimulated by opioids or NPY was altered. β-Endorphin injected icv stimulated an equivalent increase in food intake in wild-type and β-$END^{-/-}$ mice (Fig. 4A). Interestingly, the orexigenic effects of NPY were slightly increased in male β-$END^{-/-}$ mice (Fig. 4B), but were unaltered in female β-$END^{-/-}$ mice, consistent with their normal weight and feeding behavior (data not shown). NPY-stimulated food intake was inhibited equivalently by the nonselective opioid antagonist naloxone in both genotypes (Fig. 4B). Both 10 and 1 mg/kg (data not shown) naloxone attenuated NPY-induced food intake. These doses were used because they have been shown previously to attenuate food intake (22–25). Naloxone has also been shown to decrease food intake after 24-h food deprivation (23, 26, 27). We therefore tested whether this refeeding was altered in β-$END^{-/-}$ mice. However, naloxone inhibited feeding to the same extent in previously food-restricted β-$END^{-/-}$ and wild-type mice (Fig. 4C).

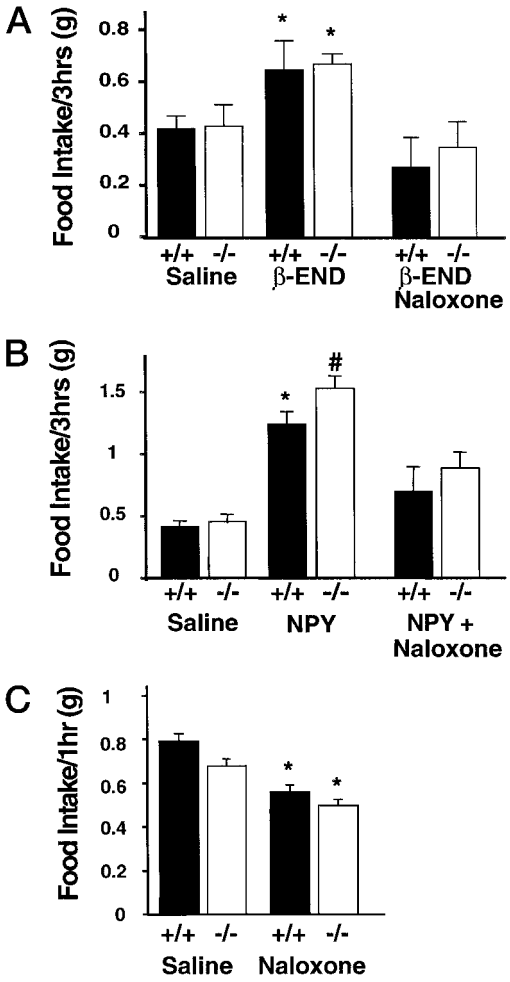

FIG. 4. Effects of β-endorphin, NPY, and naloxone on food intake in male β-$END^{+/+}$ and β-$END^{-/-}$ mice. A, Cumulative intake of food during a 3-h period after icv injection of either saline or 0.5 μg β-endorphin 15 min after an ip injection of either saline or 10 mg/kg naloxone (n = 6–10; *, $P < 0.05$ *vs.* saline, no effect of genotype, by *t* test). B, Cumulative food intake during a 3-h period after an icv injection of either saline or 1.5 μg NPY 15 min after an ip injection of either saline or 10 mg/kg naloxone (n = 10–15; *, $P < 0.01$ *vs.* saline-treated β-$END^{+/+}$ mice; #, $P < 0.01$ *vs.* saline-treated β-$END^{-/-}$ mice and $P < 0.05$ *vs.* NPY-treated β-$END^{+/+}$ mice, by *t* test). C, Food intake during a 1-h period after a 24-h fast and 15 min after an ip injection of either saline or 10 mg/kg naloxone (n = 6–10; *, $P < 0.05$ *vs.* saline, by *t* test). All data are expressed as the mean ± SEM.

Discussion

The main finding of this study was that mice lacking the endogenous opioid peptide β-endorphin have a sexually dimorphic obesity phenotype, present in only male mice. The increased adiposity of β-$END^{-/-}$ mice appears to be due to changes in caloric intake and not energy utilization. Furthermore, the effect of the opioid antagonist naloxone on food intake is unchanged in β-$END^{-/-}$ mice, suggesting that an alternative endogenous opioid tone physiologically stimulates feeding.

Our results also suggest that the effects of β-endorphin on food intake are independent of αMSH, which is proposed to be coreleased with β-endorphin from POMC neuronal terminals (6). αMSH is a potent anorexigenic peptide that plays an important role in energy homeostasis through actions primarily at the MC4 receptor (8). Here we report that the increase in food intake seen in mice lacking the MC4 receptor and that in mice lacking β-endorphin are additive, as the food intake of male mice lacking both β-endorphin and the MC4 receptor was significantly greater than that of *Mc4r*$^{-/-}$ mice. Together with our previous observations of normal tissue content of αMSH in β-$END^{-/-}$ mice (15), these data make it unlikely that the obesity phenotype of the β-$END^{-/-}$ mice is due to changes in αMSH biosynthesis or MC4 receptor signaling. We did not generate mice lacking both β-endorphin and the MC3 receptor, as the phenotype of MC3 receptor knockout mice is significantly different from that of the β-endorphin-deficient mice described here and shows a reversed sexual dimorphism (10, 11). It is therefore unlikely that the obesity phenotype of β-$END^{-/-}$ mice is due to changes in γMSH biosynthesis or MC3 receptor signaling.

POMC is expressed in both the Arc and the caudal nucleus of the solitary tract of the brainstem (28), two regions implicated in energy homeostasis (1, 4, 13). However, β-endorphin is also synthesized and released from the pituitary into the circulation, where it can act on peripheral sites to po-

tentially alter energy homeostasis (1, 4, 13, 17). Our results suggest that re-expression of β-endorphin to its central sites of release is required to fully rescue the obesity phenotype. The mechanism by which re-expression of β-endorphin to the pituitary only partially rescues the obesity is not clear. However, it may reflect either a contribution of circulating β-endorphin on peripheral sites of action or transport of β-endorphin across the blood-brain barrier and subsequent activation of central opioid receptors (29).

The obesity phenotype seen in the β-*END*$^{-/-}$ mice appears somewhat paradoxical, as exogenous opioid agonists have previously been shown to increase food intake, potentially by modulating the rewarding aspects of food (12). Indeed, we also found that exogenous β-endorphin stimulated food intake in both β-*END*$^{-/-}$ and β-*END*$^{+/+}$ mice despite the obesity resulting from the selective genetic deficiency of endogenous β-endorphin. However, a critical difference between the pharmacological and genetic manipulations of opioid action is that genetic ablation removes β-endorphin signaling from its physiological site of release only. As discussed in the introduction, this effect is impossible to mimic by pharmacological approaches. Therefore, the genetic removal of β-endorphin may reveal an anorectic effect of the peptide. Alternatively, the apparent discrepancy between the pharmacology and this genetic study could reflect the different effects of opioid agonists on short- and long-term food intake. Opioid agonists have been shown to increase short-term food intake; however, they do not increase long-term food intake (30, 31). Therefore, the genetic removal of β-endorphin may reveal a long-term anorectic action of the peptide compared with potential short-term orexigenic effects of this peptide.

Alternatively, removal of β-endorphin from the hypothalamus may disrupt normal signaling and lead to an increase in orexigenic tone. Indeed, there was a very mild increase in NPY-stimulated food intake in male, but not female, β-*END*$^{-/-}$ mice. However, the nonspecific antagonist naloxone attenuated NPY-induced food intake to the same degree in both β-*END*$^{-/-}$ and β-*END*$^{+/+}$ mice, suggesting that an endogenous opioid peptide other than β-endorphin is required for the opioid-dependent actions of NPY. Consistent with this idea of an alternative opioid tone involved in the orexigenic effects of opioids, naloxone also attenuated food intake after food deprivation to the same extent in both β-*END*$^{-/-}$ and β-*END*$^{+/+}$ mice. It is also possible that this endogenous opioid tone is a consequence of compensatory increases that occur due to the removal of β-endorphin, although we have no direct evidence for altered enkephalin or dynorphin expression in β-*END*$^{-/-}$ mice.

Recent evidence from our laboratory demonstrated that male mice lacking β-endorphin have reduced levels of operant responses (*e.g.* lever presses) for food reinforcers when maintained under *ad libitum* feeding conditions, but not when food-restricted (32). These data suggest that β-endorphin modulates the hedonic value of food independently of energy homeostasis and support a role for endogenous β-endorphin in the rewarding aspects of food intake. Together with the data presented here, these results imply multiple actions for β-endorphin in the regulation of food intake. However, as male mice lacking β-endorphin are hyperphagic and mildly obese, the overriding effect of the genetic removal of β-endorphin under *ad libitum* access to food appears to be an increase in orexigenic tone despite a reduction in the hedonic value of food intake.

β-Endorphin has been proposed to produce its feeding effects predominantly through activation of the μ opioid receptor (33), although it has affinity for μ, δ, and κ opioid receptors. No compensatory changes have been detected in the levels of μ, δ, or κ opioid receptors in β-*END*$^{-/-}$ mice (34, 35). Interestingly, *MOP*$^{-/-}$ mice have a sexually dimorphic obesity phenotype markedly similar to that of β-*END*$^{-/-}$ mice (Kieffer, B., unpublished observations). The fact that deficiencies in either the endogenous opioid ligand β-endorphin or in the μ opioid receptor cause such similar weight phenotypes indicates that the obesity, although milder than that associated with some other monogenic syndromes, reflects a real alteration in physiological function due to loss of the β-endorphin tone. Although the sexually dimorphic nature of the weight phenotype observed in β-*END*$^{-/-}$ mice remains to be fully explored, it is consistent with previous studies showing that the expression and effects of β-endorphin are sexually dimorphic (36–39).

In conclusion, β-*END*$^{-/-}$ male mice have increased weight and adiposity that appear to result primarily from increased food intake. Our results suggest the endogenous opioid β-endorphin has an unexpected anorexic effect in regulating energy homeostasis. Furthermore, these data implicate an alternative endogenous opioid system in some of the orexigenic effects of opioids previously attributed to β-endorphin.

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