Central Leptin Regulates the UCP1 and ob Genes in Brown and White Adipose Tissue via Different β-Adrenoceptor Subtypes*

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The three known subtypes of β-adrenoceptors (β1-AR, β2-AR, and β3-AR) are differentially expressed in brown and white adipose tissue and mediate peripheral responses to central modulation of sympathetic outflow by leptin. To assess the relative roles of the β-AR subtypes in mediating leptin’s effects on adipocyte gene expression, mice with a targeted disruption of the β2 adrenoceptor gene (β2-AR KO) were treated with vehicle or the β1/β2-AR selective antagonist, propranolol (20 μg/g body weight/day) prior to intracerebroventricular (ICV) injections of leptin (0.1 μg/g body weight/day). Leptin produced a 3-fold increase in UCP1 mRNA in brown adipose tissue of wild type (FVB/Nj) and β2-AR KO mice. The response was unaltered by propranolol in wild type mice, but was completely blocked by this antagonist in β2-AR KO mice. In contrast, ICV leptin had no effect on leptin mRNA in either epididymal or retroperitoneal white adipose tissue (WAT) from β2-AR KOs. Moreover, propranolol did not block the ability of exogenous leptin to reduce leptin mRNA in either WAT depot site of wild type mice. These results demonstrate that the β2-AR is required for leptin-mediated regulation of ob mRNA expression in WAT, but is interchangeable with the β1/β2-ARs in mediating leptin’s effect on UCP1 mRNA expression in brown adipose tissue.

In mice the absence of leptin (ob/ob) or its functional receptor (db/db) produces a complex metabolic syndrome characterized by hyperphagia, endocrine abnormalities, and morbid obesity (1). Deposition of excess body fat occurs even when food intake is controlled, suggesting that an important function of leptin is to regulate energy balance through modulation of metabolic efficiency. This view is supported by studies in ob/ob mice showing that leptin-injected animals lose more weight than pair-fed vehicle-injected littermates (2, 3). Of particular interest is the observation that leptin-induced weight loss occurs specifically in adipose tissue with little effect in other tissues (3, 4). The loss of adipose tissue is associated with an increase in fat oxidation, and the associated shift in fuel selection can be measured as a decrease in the respiratory quotient during leptin repletion (5, 6). Thus, adipose tissue is an important target of leptin action and the primary effect is a shift from fat storage to fat mobilization and oxidation.

This leptin-mediated shift in adipocyte function involves a coordinated change in gene expression. Two mechanisms have been postulated and include both centrally mediated effects and direct effects through functional leptin receptors (Ob-Rb) on the adipocyte (7–9). It should be noted, however, that although supraphysiologic levels of leptin are capable of producing significant direct effects on adipose tissue (10, 11), increments of plasma leptin in the physiological range are thought to act primarily through receptors in the hypothalamus (10). Occupancy of hypothalamic leptin receptors promotes activation of the sympathetic nervous system (12–15), and recent studies using surgical (16), chemical (17), and transgenic approaches (18) have shown that norepinephrine is required for leptin effects on gene expression in both brown and white adipose tissue (19–22). Thus, several lines of evidence support an emerging consensus that norepinephrine represents the peripheral signal linking hypothalamic leptin receptors to leptin-dependent changes in adipocyte gene expression.

Occupancy of each of the three known β-adrenoceptor (β-AR) subtypes leads to activation of adenyl cyclase in adipose tissue (23), but the combination of unequal expression and differing affinities for endogenous agonists has made it challenging to assess the relative contributions of each receptor subtype in various physiological states. Recent studies also demonstrate that β-adrenoceptor subtypes may be differentially coupled to various functions within the adipocyte (24–26). Therefore, we have attempted to identify the β-adrenoceptor subtype(s) that mediate the effects of leptin on gene expression in various adipose tissue depot sites. Using intracerebroventricular injections of leptin in mice lacking β1- or β3-adrenoceptors, we show that different complements of β-β-adrenoceptor subtypes are required to transduce leptin’s effects on gene expression in white versus brown adipose tissue.

EXPERIMENTAL PROCEDURES

Experimental Animal Protocol—Mice in each of the four experiments described below were housed in pairs in solid-bottom cages with continuous access to chow (Purina Mouse Chow, Ralston Purina, St. Louis, MO) and water. Room temperature was maintained at 22–23 °C, and the lights were on a 12-h light/dark cycle. The animals were acclimated for 1–2 weeks prior to each study, and injected thereafter with various agents according to protocols specified under each experiment. All in...
Experiments were given 2 h following the start of the light cycle, and all mice were sacrificed 2 or 4 h after the last injection in the series. Thereafter, interscapular BAT, as well as epididymal, retroperitoneal, and inguinal WAT depots were carefully removed, weighed, and used for preparation of total RNA or isolation of adipocytes.

Intraperitoneal Injections—Mice were anesthetized by inhalation of isoflurane and a guarded, blank 27-gauge 0.5-inch needle was used to create a guide injection site 0.7 mm posterior to bregma and 1.0 mm lateral to midline at a depth of 4.0 mm (27). In the experiments proper, a 10.0-μl Hamilton 1700 series gastight syringe (Hamilton, Reno, NV) was used to inject artificial cerebrospinal fluid (aCSF), murine leptin, or rat neuropeptide Y in a volume of 2–5 μl. Correct positioning of the guide injection site was confirmed prior to the start of each experiment by monitoring feeding behavior following injection of neuropeptide Y (0.075 μg/g body weight). Mice failing to respond to neuropeptide Y were removed from the experiment. aCSF, consisting of 70 mM NaCl, 6 mM KCl, 0.7 mM CaCl₂, 0.85 mM MgCl₂, 0.75 mM Na₃HPO₄, 0.10 mM NaH₂PO₄, and 0.1% untreated bovine serum albumin, was injected in a volume of 5 μl. Thereafter, mice were monitored to ensure full recovery.

Experiment 1—Seven-week-old male C57BL/6J mice were obtained from Jackson Laboratories (Bar Harbor, ME) and acclimated for 2 weeks prior to the study. Thereafter, mice were injected ICV with leptin (0.1 μg/g body weight/day) for 1, 2, or 3 days, and representative mice were sacrificed 2 and 4 h following the respective injections on each day. Separate mice receiving vehicle injections were sacrificed 2 h after injection. Control mice were injected ICV with aCSF. Interscapular BAT, as well as epididymal, retroperitoneal, and inguinal WAT depots were removed, weighed, and used to prepare total RNA as described previously (19).

Experiment 2—Seven-month-old male FVB/NJ (WT) mice and age-matched FVB/NJ male mice with a targeted disruption of the β₃-adrenergoreceptor (β₃-AR KO) gene (28) were acclimated as described above and equal numbers of each phenotype were randomly assigned to one of four treatment groups. The mice in group 1 received ICV injections of aCSF for 3 days, and food was provided ad libitum. Mice in group 2 received ICV injections of mouse leptin (0.1 μg/g body weight/day) for 3 days and were pair-fed to the mean intake of mice in groups 2 and 3. Two h after the final injection, the mice were sacrificed, and the adipose tissue depot sites were carefully removed and used for cell isolation and membrane preparation as described below.

Experiment 3—Male C57BL/6J (ob/ob) mice and their lean littermates were obtained from Jackson Laboratories at 6 weeks of age and randomly assigned to one of two treatment groups. The mice were housed individually at 23 °C and equilibrated for 4 days before beginning the experiment. On the morning of the 5th day and for 2 mornings thereafter, half the mice in each phenotype received intraperitoneal injections of recombinant mouse leptin (20 μg/g body weight/day), while the remaining mice in each phenotype received vehicle injections. Within each phenotype, the mice receiving vehicle were pair-fed with the mice receiving leptin. Three h following the final injections on day 7, the mice were weighed, sacrificed, and adipose tissue processed as described previously.

Experiment 5—Seven-month-old male FVB/NJ mice and age-matched β₃-AR KO male mice were acclimated as described above. Thereafter, the WT and β₃-AR KO mice were sacrificed, and the adipose tissue depot sites were carefully removed and used for cell isolation and membrane preparation as described below.

Materials—All reagents, except where noted, were obtained from Sigma and were of the highest reagent grade. T1-RNase and Trizol LS reagent were from Life Technologies, Inc. Oligonucleotide primers and DNA sequencing were generated at the DNA Core Facility at the Medical University of South Carolina. [3H]-cAMP and [3H]diphenylhexylamine were purchased from PerkinElmer Life Sciences. 1-Chloro-2,2,2-trifluoroethyl difluoromethyl ether (isoflurane) was from Ohmeda (Danbury, CT). Adenylate cyclase activity was determined in adipocyte membranes using combinations of receptor subtype selective agonists and antagonists to assess the functional activity of each receptor subtype (31, 32, 35). The β₁-AR antagonist, SR 59230A (36) was obtained from Dr. Luciano Manara (Sanofi Midy, Milan, Italy) and solubilized in ethylene glycol at 200 μg/ml. The antagonist was used at a final concentration of 10 μM as reported previously (36). Other agonists and antagonists were from commercial sources.

Methods of Analysis—One-way analysis of variance was used to compare group means for UCPI mRNA, leptin mRNA, β₁-AR mRNA, β₁-AR mRNA, β₂-AR mRNA, β₂-AR mRNA, adenyl cyclase activity, and ICYP binding in the respective experiments for each variable. The level of protection against type I errors was set at 5%, and P values for specific treatment comparisons are presented under “Results.”

RESULTS

Time Course of Central Leptin Effects on BAT UCPI in C57BL/6J Mice—To determine the time course for leptin’s effects on UCPI expression in BAT and distinguish between central versus peripheral effects of the peptide, mice were given ICV injections of leptin or artificial CSF and sacrificed 2 and 4 h later on successive days. This protocol complemented our normal treatment regimen of 3 days and revealed that leptin increased UCPI mRNA 2 and 4 h after the initial injection on day 1 (Fig. 1). The −2-fold increase was maximal 4 h after
duced basal expression of UCP1 mRNA in WT mice, but did not follow injection as described under “Experimental Procedures.” The relative abundance of UCP1 mRNA was quantitated by comparing the densitometric intensities of protected fragments from each treatment group to known amounts of sense strand transcripts that were hybridized simultaneously. Individual RNA samples from each animal were analyzed to calculate group means, and representative samples are presented in the figure (aCSF, 1.71 ± 0.04 fmol of UCP1 mRNA/µg of RNA, n = 3; leptin at 2 h, 2.32 ± 0.16 fmol of UCP1 mRNA/µg of RNA, n = 6; leptin at 4 h, 2.94 ± 0.29 fmol of UCP1 mRNA/µg of RNA, n = 6).

**Fig. 1. Ribonuclease protection assay of UCP1 mRNA from BAT of C57BL/6J mice.** 0.5 µg of total RNA from BAT was hybridized with an antisense probe for UCP1 mRNA (nucleotides 7–300) and 18 S rRNA (nucleotides 715–794). Mice were injected ICV with either aCSF (5 µl) or leptin (Lep, 2 µg) for 1 day and sacrificed either 2 or 4 h following injection as described under “Experimental Procedures.” The relative abundance of UCP1 mRNA was quantitated by comparing the densitometric intensities of protected fragments from each treatment group to known amounts of sense strand transcripts that were hybridized simultaneously. Individual RNA samples from each animal were analyzed to calculate group means, and representative samples are presented in the figure (aCSF, 1.71 ± 0.04 fmol of UCP1 mRNA/µg of RNA, n = 3; leptin at 2 h, 2.32 ± 0.16 fmol of UCP1 mRNA/µg of RNA, n = 6; leptin at 4 h, 2.94 ± 0.29 fmol of UCP1 mRNA/µg of RNA, n = 6).

**Effect of Leptin on BAT UCP1 in β3-KO Mice**—The purpose of experiment 2 was to determine whether the absence of the β3-AR in both BAT and WAT would compromise the ability of centrally administered leptin to regulate gene expression in each depot site. A secondary objective was to determine whether the β1/β2-ARs could substitute for the missing β3-AR or whether α-adrenergic receptors might also be involved in mediating the response. WT age-matched FVB/NJ mice served as positive controls and responded to ICV-injected leptin with a 4–5-fold increase in UCP1 mRNA in BAT (p < 0.01, Fig. 2A). Intraperitoneal injection of propranolol to block β1/β2-ARs reduced basal expression of UCP1 mRNA in WT mice, but did not block leptin’s ability to induce UCP1 mRNA expression by 4-fold (p < 0.01, Fig. 2A). These results suggest that the β1/β2-ARs and β3-ARs are interchangeable with respect to their ability to mediate central effects of leptin on UCP1 expression. This conclusion is supported by results from the β3-AR KO mice, which show that central leptin produced a β3-4-fold increase in UCP1 mRNA (p < 0.01, Fig. 2B), and the finding that propranolol completely blocked the ability of leptin to increase UCP1 mRNA in BAT from these animals (p < 0.01, Fig. 2B). These findings also make the case that α-adrenergic receptors are not involved in the response and that β-adrenergic receptors are the primary adrenergic receptors mediating the effects of leptin on UCP1 mRNA.

**Effect of ICV Leptin on Leptin mRNA in WAT**—To test the hypothesis that centrally administered leptin regulates its own expression in WAT, we measured leptin mRNA in epididymal and retroperitoneal depot sites from WT and β3-AR KO mice treated with leptin. The two sites were chosen as being representative of WAT depots that respond differently to adrenergic stimulation (19). In WT mice, ICV leptin produced a highly significant reduction (p < 0.01) of leptin mRNA in epididymal WAT from 0.021 ± 0.003 fmol of leptin mRNA/µg of RNA to 0.005 ± 0.002 fmol/µg of RNA (Fig. 3A). A similar reduction in leptin mRNA (p < 0.001) was noted in the retroperitoneal WAT depot of these mice (Fig. 4A). Figs. 3A and 4A show that propranolol produced a modest increase in basal leptin mRNA in both depot sites of WT mice (p < 0.05), but did not impair leptin’s ability to decrease expression of its own message in either site (Figs. 3A and 4A). In contrast, ICV leptin failed to decrease leptin mRNA in epididymal or retroperitoneal WAT from β3-AR KO mice (Figs. 3B and 4B). Propranolol alone produced a slight increase in leptin mRNA in both depot sites of β3-AR KO mice (p < 0.05), and treating these mice with propranolol and leptin produced no additional change in leptin mRNA (Figs. 3B and 4B). The failure of ICV leptin to down-regulate leptin mRNA in WAT from β3-AR KO mice coupled with the inability of propranolol to block leptin's ability to down-regulate leptin mRNA in WT mice indicates that the β3-adrenoceptor is both necessary and sufficient for mediation of this response.

**Additional data** that support the importance of the β3-AR in...
regulating leptin expression can be found by comparing leptin mRNA and serum leptin in WT and β₂-AR KO mice. If our hypothesis is correct, the absence of the β₂-AR should lead to up-regulation of leptin mRNA. The data from epididymal WAT support this conclusion by showing that leptin mRNA is 3-fold higher (p < 0.01) in the β₂-AR KO mice compared with WT mice (WT (0.021 ± 0.003 fmol of leptin mRNA/μg of RNA) versus β₂-AR KO (0.072 ± 0.005 fmol/μg of RNA)). The difference was even more striking in the retroperitoneal depot, where leptin mRNA was 5-fold higher in β₂-AR KO compared with WT mice (WT (0.012 ± 0.004 fmol/μg of RNA) versus β₂-AR KO (0.070 ± 0.009 fmol/μg of RNA)). Circulating leptin levels were also higher (p < 0.05) in β₂-ARKOs (8.7 ± 0.4 ng/ml) compared with WT mice (5.6 ± 0.4 ng/ml), although the magnitude of the difference was less than that noted with leptin mRNA. Taken together, the results support the conclusion that the absence of the β₂-AR leads to up-regulation of leptin expression.

**Existence and Functionality of β₁- and β₃-AR in FVB/NJ and β₂-AR KO Mice**—The results from experiment 2 support the conclusion that there are tissue-specific differences in the requirement for the β₂-AR in mediating leptin effects on gene expression. This interpretation is based on the assumption of comparable expression patterns and functionality of the β₁/β₃-ARs in BAT and WAT between the WT and β₂-AR KO mice. To address this question, we compared β₁- and β₃-AR mRNA levels and ICYP binding to β₁- and β₃-ARs in adipose tissue from both phenotypes. Fig. 5 shows that mRNA levels of both β₁- and β₃-AR mRNA are reduced 2-fold (p < 0.01) in BAT of β₂-AR KO mice compared with WT mice (β₂-AR WT, 0.036 ± 0.008 fmol/μg of RNA; β₂-AR KO, 0.013 ± 0.006 fmol/μg of RNA). In epididymal WAT (Fig. 5), a similar 2-fold decrease (p < 0.05) in β₁-AR mRNA was noted in β₂-AR KO mice (0.019 ± 0.002 fmol/μg of RNA) compared with WT mice (0.041 ± 0.003 fmol/μg of RNA). In contrast, β₂-AR mRNA was significantly increased (p < 0.05) in β₂-AR KO mice (0.178 ± 0.024 fmol/μg of RNA).
WT FVB/NJ (18.6 ± 6 levels in this group (Fig. 5) compared with the WT mice.

Thus, the modest decrease in binding in BAT membranes was similar between WT FVB/NJ and β3-AR KO, 0.019 ± 0.002 fmol/μg of RNA, n = 4; β3-AR KO, 0.013 ± 0.006 fmol/μg of RNA, n = 3. BAT, β1-AR mRNA WT, 0.041 ± 0.003 fmol/μg of RNA, n = 3; β3-AR KO, 0.019 ± 0.002 fmol/μg of RNA, n = 4; β2-AR mRNA WT, 0.009 ± 0.003 fmol/μg of RNA, n = 4; β2-AR KO, 0.178 ± 0.024 fmol/μg of RNA, n = 4).

To evaluate whether the observed changes in mRNA levels are reflected by comparable changes in β1-AR and β2-AR binding capacity, we used a competition radioligand binding approach with ICYP in the presence of the selective β3-AR agonist, CL316,243, to eliminate low affinity binding of ICYP to β3-ARs (37). Then, using crude membrane preparations from brown and white adipocytes of each phenotype, the highly selective β1-AR antagonist, CGP-20712A, which displays ~1000-fold selectivity for β1-AR over β2-AR (38), was used to resolve ICYP binding into the components contributed by the β1- and β2-AR subtypes (Fig. 6, A and B). With ICYP at 30 pm (Fig. 6A), total ICYP bound by both β1- and β2-ARs in white adipocyte membranes was similar between WT FVB/NJ (21.3 ± 0.5 fmol/mg of protein) and β3-AR KO mice (19.7 ± 0.3 fmol/mg of protein). The high affinity binding component of the curves, resolved by curve fitting and defined as the β1-AR, accounted for 28% (5.6 ± 0.6 fmol/mg) of the total binding sites in membranes from WT FVB/NJ mice. In β3-AR KO mice, the high affinity component accounted for 19% (3.8 ± 0.5 fmol/mg) of total ICYP binding (Fig. 6A). The second component of the curves represents ICYP bound by the β2-AR and represents the remaining binding sites (WT FVB/NJ, 74%; β3-AR KO, 81%). Although the total ICYP binding to white adipocyte membranes did not differ between the two groups, comparison of the respective binding curves using an F test indicated that the proportion of binding sites contributed by the β1- and β2-AR subtypes in each group was significantly different (p < 0.01).

Thus, the modest decrease in β1-AR binding sites and increase in β2-AR binding sites noted in the β3-AR KO mice was statistically significant (Fig. 6A) and consistent with the observed changes in β1- and β2-AR mRNA levels in this group (Fig. 5) compared with the WT mice.

In the case of BAT, total ICYP binding did not differ between WT FVB/NJ (16.5 ± 0.3 fmol/mg) and β3-AR KO mice (17.5 ± 0.4 fmol/mg, Fig. 6B). The high affinity β1-AR component comprised 27% (5.0 ± 0.6 fmol/mg) of total binding sites in WT FVB/NJ membranes and 28% (4.9 ± 0.4 fmol/mg) of total binding sites in the β3-AR KO group (Fig. 6B). The remaining β2-AR binding sites comprised 73% and 72% of total ICYP binding in BAT membranes from WT and β3-AR KO mice, respectively. In contrast to BAT, where subtle alterations were noted, we found no evidence of a change in the proportion of β1- or β2-AR binding sites in BAT between the groups (Fig. 6B). In contrast to BAT, where binding and mRNA results were consistent, the similarity in binding capacity was not reflective of the observed group differences in BAT β1- and β2-AR mRNA levels (Fig. 5).
**Differential Regulation of UCP1 and Leptin by β-Adrenoceptors**

**TABLE I**

Relative contributions of β-adrenergic subtype to activation of adenylyl cyclase in brown and white adipocyte membranes from control and β₃-adrenoceptor knockout mice

| Assay components | Adenylylcyclase activity | Control mice | β₂-AR knockout mice |
|------------------|--------------------------|--------------|---------------------|
|                  | BAT                      | WAT          | BAT                 | WAT                 |
|                  | pmol cAMP/min/mg         | pmol cAMP/min/mg |
| Basal            | 19.1 ± 1.4               | 44.9 ± 1.9   | 19.7 ± 3.4          | 47.1 ± 4.0          |
| SR59230A        | 21.2 ± 3.3               | 44.7 ± 2.1   | 23.3 ± 3.3          | 41.5 ± 4.4          |
| Propranolol      | 16.3 ± 1.2               | 44.7 ± 3.0   | 20.4 ± 2.2          | 41.1 ± 3.9          |
| Isoproterenol   | 135 ± 17                 | 210 ± 10     | 83.2 ± 12.2         | 63.9 ± 4.4          |
| Isoproterenol + SR59230A | 59 ± 4.7            | 61.6 ± 2.6   | 85.3 ± 9.1          | 59.4 ± 5.0          |
| Isoproterenol + propranolol | 106 ± 12            | 181 ± 15    | 47.3 ± 5.1          | 51.0 ± 3.4          |
| Epinephrine      | 86.4 ± 15.3              | 62.7 ± 2.7   | 71.4 ± 2.8          | 63.7 ± 4.0          |
| Epinephrine + propranolol | 39.5 ± 1.9             | 47.5 ± 3.3   | 34.4 ± 2.1          | 48.5 ± 3.7          |
| Forskolin        | 364 ± 58                 | 562 ± 46     | 335 ± 43            | 474 ± 61            |

*The β₂-adrenergic antagonist SR59230A (36) was used at 10 μM to selectively block β₂-adrenergic activation. Propranolol was used at 100 nM to fully activate β₁- and β₂-adrenocorticotropic hormones, respectively (Table I). A similar assessment of receptor subtype contribution was reached with epinephrine, which was used at 1 μM to fully activate the β₁- and β₂-adrenocorticotropic hormones without activating the β₃-adrenocorticotropic hormone. Forskolin was used at 100 μM to assess the total adenylyl cyclase catalytic activity in adipocyte membranes from each tissue type and genotype.*

Functionality of β₁- and β₂-ARs in Adipocyte Membranes of FVB/NJ and β₃-KO Mice—The third component of studies in experiment 3 was to assess the functional coupling of β₁- and β₂-ARs to AC in adipocyte membranes from the two groups. The goal of these studies was to complement the binding studies by testing the hypothesis that expressed β-adrenoceptor subtypes are equivalently coupled to AC in each group. Clarification of this point would rule out altered expression or effector coupling of β₁- or β₂-ARs as the basis for the differential requirements of receptor subtypes in regulating gene expression between BAT and WAT that was shown in experiment 2. To that end, combinations of nonselective and selective receptor agonists and antagonists were used in a systematic attempt to evaluate the relative contributions of the β₁/β₂-ARs and β₃-ARs in activating AC in adipocyte membranes from each group. As shown in Table I, basal AC activity in white and brown adipocyte membranes did not differ between WT and β₃-AR KO mice. As judged by forskolin stimulation, total AC activity in BAT and WAT was also unaffected by the absence of the β₃-AR (Table I). The selective β₃-AR antagonist, SR59230A, and the selective β₁/β₂-AR antagonist, propranolol, were used in combination with isoproterenol to show that the β₁/β₂-ARs accounted for between 10% and 30% of the total AC activation elicited by isoproterenol in BAT and WAT membranes from WT mice, respectively (Table I). A similar assessment of receptor subtype contribution was reached with epinephrine, which was used at 1 μM to fully activate the β₁- and β₂-ARs without significantly activating the β₃-AR (Table I). Using both strategies, AC activation attributable to the β₁/β₂-ARs in membranes from WT mice was similar to the stimulation of AC activity in β₃-AR KO mice that is solely attributable to the β₁/β₂-ARs (Table I). Taken together, these data establish the presence and functionality of β₁- and β₂-ARs on brown and white adipocytes in β₃-AR KO mice at levels that are comparable to those in WT mice.

Effect of Leptin on Leptin mRNA in ob/ob Mice—A practical test of our conclusion that the β₃-AR is required for leptin-dependent regulation of leptin mRNA in WAT is that ob/ob mice. This follows from our previous demonstration that expression of the β₃-AR is severely compromised in both brown and white adipocytes from ob/ob mice (35). Ob/ob mice are also particularly well suited for this purpose because of the absence of functional leptin and the resulting sensitivity to leptin replacement. Results from experiment 4 show that treatment of ob/ob mice for 3 days with leptin failed to reduce leptin mRNA in either retroperitoneal or epididymal WAT (Fig. 7). Similar treatment of lean mice produced a 6–7-fold reduction in leptin mRNA in both sites (Fig. 7 and Table II). The reduction in β₃-AR mRNA in WAT from the ob/ob mice used here was on the order of 50–100-fold (Table II). These findings are consistent with and support our conclusion that the β₃-AR is required for leptin-dependent regulation of leptin mRNA in WAT. To test whether the requirement for the β₃-AR is specific to WAT or specific to the gene being regulated, we examined the effect of leptin in BAT from the same mice. Expression of the β₃-AR is also compromised in BAT from ob/ob mice (Table II), so if the effect is dependent on the β₃-AR, regardless of site, we would predict that leptin mRNA would not be reduced by leptin in BAT. Quite the contrary, BAT from the same ob/ob mice showed a significant reduction in leptin mRNA following leptin treatment (vehicle, 0.020 ± 0.001 fmol/μg of RNA; leptin, 0.007 ± 0.001 fmol/μg of RNA). Collectively, these results indicate that regulation of leptin expression requires the β₃-AR in BAT but is capable of using the β₁/β₃-AR in BAT.

Effect of Cold Exposure on Adipocyte Gene Expression in β₃-AR KO Mice—A more general test of our hypothesis that the sympathoexcitatory nervous system utilizes different complements of β-adrenoreceptors to mediate the effects of norepinephrine in white versus brown adipose tissue can be made by exposing β₃-AR KO mice to cold. In experiment 5, the β₃-AR KOs adapted to cold exposure readily and increased UCP1 mRNA to the same extent as their WT controls (data not shown). This finding agrees with observations made by the original developers of this transgenic mouse line (28), but of particular interest is our finding that cold exposure does not reduce leptin mRNA in WAT of β₃-AR KO mice (room temperature, 0.056 ± 0.009 fmol/μg RNA; 4°C, 0.094 ± 0.015 fmol/μg RNA). In contrast, 4 h of cold exposure in WT mice reduced leptin mRNA to the detection limit of the assay (data not shown). Taken together, these studies show that the β₁/β₂-ARs are required to transduce the effects of changes in sympathetic outflow on leptin expression in WAT. As noted with ICV leptin, the β₁/β₂-AR is capable of substituting for the β₃-AR in mediating sympathoexcitatory effects of leptin on gene expression in BAT.

**DISCUSSION**

In the present study, we have used the ICV route of administration to study centrally-mediated effects of leptin on gene
expression in adipose tissue. The technique was used with mice lacking \( \beta_3 \)-adrenoreceptors (\( \beta_3 \)-AR KO) to evaluate the involvement of this and other adrenoreceptor subtypes in mediating peripheral effects of leptin. This experimental approach is based on an emerging consensus that leptin regulates adipocyte function through central modulation of the sympathetic nervous system. The initial evidence came from a study showing that leptin increased norepinephrine turnover in BAT (12), and was followed by studies mapping the neural pathways within the sympathetic nervous system activated by leptin (13–15). Subsequent studies have shown that surgical (16), chemical (17, 39), or genetic (18) sympathectomy blocked leptin’s effects on gene expression in both BAT and WAT. The latter studies established a requirement for the long form of the leptin receptor (Ob-Rb), but did not distinguish between central versus peripheral sites of action for leptin. The requirement of norepinephrine (18) suggested that central leptin receptors were activated, but the involvement of peripheral leptin receptors that communicate with the brain by afferent autonomic nerves (40, 41) could not be excluded. Results from the present study argue that peripheral leptin receptors are not required for leptin’s effects on adipocyte gene expression, and make the case that central leptin receptors mediate the observed effects on UCP1 mRNA in BAT and leptin mRNA in WAT. Additionally, based on the comparable magnitude of the effects produced by central versus peripheral administration of leptin in our studies, it seems reasonable to assume that peripherally injected leptin is primarily working through central leptin receptors. Taken together, the evidence is compelling that leptin regulates adipocyte gene expression through central modulation of sympathetic nervous system outflow. The premise that norepinephrine is the peripheral mediator of leptin action is the basis for using the present experimental approach to deduce which adrenoreceptor(s) mediate the respective responses in brown and white adipose tissue.

Several recent studies have suggested the existence of a putative \( \beta_3 \)-AR based on interpretation of pharmacological profiles from adipocytes expressing the \( \beta_3 \)-AR at various levels (42–44). The existence of a fourth \( \beta \)-adrenoreceptor could complicate interpretation of the present experiments, which assume the existence of only three \( \beta \)-adrenoreceptors. The evidence for a \( \beta_3 \)-AR centers around the observation that CGP12177A, a partial agonist of the \( \beta_3 \)-AR and antagonist of \( \beta_1/\beta_2 \)-ARs, activates lipolysis in adipocytes containing little or no \( \beta_2 \)-AR (42, 43). Complementing this result is the observation that \( \beta_2 \)-AR-selective or nonselective \( \beta \)-AR antagonists block this response while the \( \beta_2 \)-AR-selective antagonist, SR59230, does not (43). It should be noted, however, that the documented heterogeneity in the relationship between binding affinity and coupling efficiency with ligands for \( \beta \)-adrenoreceptor subtypes complicates interpretation of pharmacological approaches in adipocytes (45–49). Such seems possible with CGP12177 after the careful studies of Konkar et al. (50) have systematically documented an unexpected activation of the \( \beta_3 \)-AR by this ligand. Thus it is unnecessary to invoke the existence of a \( \beta_3 \)-AR to explain the results obtained with CGP12177 if its binding to the \( \beta_3 \)-AR results in partial activation. In the present study, conclusions regarding requirements for specific \( \beta \)-receptors in WAT and BAT are not predicated on the existence of the putative \( \beta_3 \)-AR because of the adequacy of a model containing \( \beta_1 \), \( \beta_2 \), and \( \beta_3 \)-ARs in explaining the observed results.

The most significant finding from the present study is the differential requirement for the \( \beta_3 \)-AR in mediating leptin’s effects in brown versus white adipose tissue. The concept of differential coupling of \( \beta \)-adrenoreceptor subtypes to various responses within the same cell is not new. The subject has received significant attention in BAT, where densely innervated brown adipocytes are stimulated by norepinephrine in response to environmental and physiological stimuli (12, 51). Using isolated brown adipocytes from both Syrian hamsters (52) and rats (24, 53), Zhao et al. addressed the role of \( \beta_3 \)-ARs versus \( \beta_1 \)-ARs in mediating catecholamine-stimulated oxygen consumption. Based on analysis of Schild plots constructed from propranolol-mediated inhibition of norepinephrine dose-response curves, the authors concluded that the thermogenic response was coupled solely to the \( \beta_3 \)-AR (24, 53). This conclusion is at odds with results from experiments with \( \beta_3 \)-AR knockouts where thermogenic responses to norepinephrine, isoproterenol, and cold exposure were normal (28). Thus, the absence of the \( \beta_3 \)-AR did not compromise the ability of BAT to respond to catecholamines. A role for the \( \beta_1 \)-AR is also supported by the work of Atigie et al. (25), who used isolated brown adipocytes to show that a specific \( \beta_1 \)-AR antagonist effectively blocked the effects of low concentrations of norepinephrine (25–100 nm) on respiration. The authors concluded that, within the physiological range of norepinephrine concentrations, the \( \beta_1 \)-AR makes a significant contribution to activating respiration. Chaudhry and Granneman (26) investigated the possibility that \( \beta_3 \)-ARs and \( \beta_2 \)-ARs serve different signaling functions in brown adipocytes. Supported by data showing the predicted compartmentalization of phosphorylated proteins (cAMP-responsive element-binding protein and perilipin), the authors concluded that norepinephrine induced UCP1 expression preferentially through the \( \beta_1 \)-AR and lipolysis primarily through the \( \beta_3 \)-AR (26). Our results extend these findings to the intact animal and show for the first time that centrally mediated effects of leptin on UCP1 expression in brown adipose tissue can be transduced interchangeably by \( \beta_1/\beta_2 \) or \( \beta_3 \)-ARs. Our results demonstrate that this conclusion is not an artifact of altered expression or function of the \( \beta_1/\beta_2 \)-ARs in BAT from \( \beta_3 \)-AR KO mice. The present findings are also consistent with the original report with these mice showing comparable cold-induced increases in UCP1 mRNA in BAT from wild type and \( \beta_3 \)-AR KO mice (28). A study by Revelli et al. (54) using C57BL/6J mice with the \( \beta_2 \)-AR gene deleted by homologous recombination found a positive correlation between UCP1 and \( \beta_3 \)-AR mRNA, indicating a role for the \( \beta_3 \)-AR in the absence of the \( \beta_3 \)-AR. It should also be noted that, despite significant reductions in \( \beta_3 \)-AR expression in BAT from \( \beta_3 \)-AR KO mice (35),
they respond to leptin repletion with robust increases in BAT UCP1 expression (19). Taken together, the evidence is internally consistent and supports our conclusion of interchangeability of receptor subtypes in this tissue.

In contrast to BAT, where the β3-AR is sufficient but not necessary to mediate leptin effects on gene expression, the present work makes the case that the β3-AR is required for inhibitory regulation of leptin mRNA in WAT. Four lines of evidence support this conclusion. First, norepinephrine is required for inhibitory regulation of leptin mRNA by leptin (18). Our previous work made the case that the requirement for the β3-AR is specific to the gene being regulated. One way to address this question arising from the present work is whether the requirement for the β3-AR in WAT is targeted to a compartment that is necessary for inhibitory regulation of leptin expression. If so, this would be consistent with the observations of Chaudhry and Granneman (26), who reported differential targeting of phosphorylated proteins to subcellular compartments produced by β3- versus β2-AR activation in brown adipocytes (26). It will be interesting to determine whether the morphological differences between brown and white adipocytes are responsible for the differential coupling of adrenoreceptors to gene expression in the two cell types, and whether the differences described here extend to other genes regulated by leptin in the two types of adipocytes.

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TABLE II

| Gene/tissue | Lean (n = 4) | Leptin (n = 5) | Saline (n = 6) | Leptin (n = 6) |
|-------------|-------------|---------------|-------------|---------------|
| Leptin/ EWAT | 0.074 ± 0.011 | 0.011 ± 0.003* | 0.281 ± 0.041 | 0.324 ± 0.039 |
| Leptin/ RPAT | 0.056 ± 0.009 | 0.009 ± 0.002* | 0.182 ± 0.028 | 0.237 ± 0.031 |
| Leptin/ IBAT | 0.014 ± 0.004 | 0.004 ± 0.001* | 0.020 ± 0.001 | 0.007 ± 0.001* |

* Indicates a significant difference (P < 0.01) from saline-treated animals within genotypes.
