The role of the sympathetic nervous system in the regulation of leptin synthesis in C57BL/6 mice

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Abstract The objectives of this study were to determine whether leptin synthesis is regulated by the sympathetic nervous system and if so whether β-adrenergic receptors mediate this effect. We show that sympathetic blockade by reserpine increases leptin mRNA levels in brown but not white adipose tissue, while acute cold-exposure decreases leptin expression 10-fold in brown adipose tissue and 2-fold in white adipose tissue. The cold-induced reduction in leptin mRNA can be prevented by a combination of propranolol and SR 59230A but not by either antagonist alone, indicating that β_3-adrenergic receptors and classical β_1/β_2-adrenergic receptors both mediate responses to sympathetic stimulation. Circulating leptin levels reflect synthesis in white adipose tissue but not in brown adipose tissue.

Key words: Leptin; β_3-Adrenoceptor; β_1/β_2-Adrenoceptor; Sympathetic nervous system

1. Introduction

Obesity results from a chronic disequilibrium between caloric intake and energy expenditure, and is associated with an increased risk for diabetes and cardiovascular disorders. Energy expenditure is determined partly by activity and exercise, and partly by adaptive thermogenesis. This process is driven by the sympathetic nervous system (SNS) and occurs in tissues expressing uncoupling proteins (UCP-1,-2 and -3), including brown adipose tissue (BAT) and skeletal muscle. One mediator of thermogenesis is the hormone leptin, which plays a critical role in energy balance by reducing food intake as well as increasing energy expenditure [1]. Leptin infusion has been shown to increase expression of UCP-1 in BAT, but only in the presence of an intact sympathetic innervation [2]. Leptin has also been shown directly to increase sympathetic nerve activity in BAT and other regions including rat hindlimb [3].

Leptin is synthesised primarily in white adipose tissue (WAT), and to a lesser extent in BAT [4,5]. The synthesis of leptin is regulated by both chronic and acute stimuli. Obese animals and humans have high levels of leptin mRNA and circulating leptin, due to stimulation of leptin expression with increasing size of white adipocytes [6]. In the short term however, leptin mRNA is modulated by feeding status, plasma insulin and corticosteroid levels [7,8]. There is considerable evidence that a negative feedback loop involving the sympathetic nervous system also regulates leptin synthesis. When the SNS is stimulated by acute cold-exposure, a substantial reduction in leptin mRNA has been observed in both WAT [9] and BAT [10]. Conversely, blockade of noradrenaline synthesis by α-methyl-*p*-tyrosine has been shown recently to cause marked increases in WAT leptin mRNA and also plasma leptin [11]. The effect of cold-exposure can be mimicked by treatment of animals with β-adrenoceptor (AR) agonists [9,10,12,13]. This is a direct effect on adipocytes, as the suppression of leptin synthesis by β-AR agonists is seen in isolated brown [5] and white adipocytes [14,15]. Inhibition of leptin expression also occurs following treatment of cells with cyclic AMP (cAMP) analogues and forskolin, indicating involvement of the cAMP/protein kinase A signalling pathway [5,14,16].

It is widely supposed that the β_3-AR mediates the effect of the SNS on leptin expression [5,11,13,14,17]. The present study describes the effects of modulating SNS activity on the expression of leptin mRNA in WAT and BAT and on levels of circulating leptin. Using the antagonists propranolol and SR 59230A [18], we show that β_3-ARs are not solely responsible for mediating the effects of endogenous sympathetic stimulation, with β_1/β_2-ARs also playing an important role.

2. Methods

2.1. Animals and treatments

All animals used were 8 weeks old male C57BL/6J lean (+/+) mice. Mice had continuous access to tap water and a standard rodent diet containing 20%(w/w) protein and 3%(w/w) fat. Mice were anaesthetised by 80% CO_2/20% O_2 and killed by decapitation (according to guidelines produced by the Monash University Animal Experimentation Ethics Committee). Blood was collected into tubes containing 50 U heparin, and plasma was harvested by centrifugation and stored at −70°C until analysis. Circulating leptin was measured by a commercial leptin RIA kit (Linco Research, St. Charles, MO, USA) with a limit of sensitivity of 0.2 ng/ml. Epididymal WAT and interscapular BAT, carefully freed from surrounding WAT, were dissected and stored at −70°C until analysis.

Reserpine was used to investigate the effects of sympathetic blockade. Reserpine was dissolved in 20% ascorbic acid solution, the 20% ascorbic acid alone being administered i.p. to control animals. Treated mice received 2 mg/kg i.p. at time 0, with a second dose of 2 mg/kg i.p. at 20 h. The mice were killed 24 h after the second injection. To investigate the effects of acute cold-exposure, mice were housed individually in empty plastic cages and placed in a cold room at 4°C for 4 h. Control animals remained at room temperature (22°C). The effects of β-AR antagonists were also investigated by injecting 1.5 mg/kg SR-59230A i.p., 1.5 mg/kg (−)-propranolol i.p. or both antagonists together at room temperature 15 min before the mice were placed at 4°C or left at 22°C for 4 h. Both the SR-59230A and (−)-propranolol were dissolved in 5%(v/v) dimethyl sulphoxide/2.5%(v/v) ethanol. This solvent (0.1 ml) was administered i.p. to control mice.

2.2. Preparation of RNA

RNA was isolated from tissues using a procedure based on that of Chomczynski and Sacchi [19]. Frozen tissue was ground to a fine powder in a stainless steel mortar and pestle pre-cooled in liquid nitrogen. Total RNA was extracted by homogenising in TRIZOL

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according to the manufacturer's instructions. To avoid cross-contamination, the homogeniser probe was dismantled and washed thoroughly between each sample. The yield and quality of the RNA were assessed by measuring absorbance at 260 and 280 nm, and by electrophoresis on 1.2% agarose gels.

2.3. Reverse transcription (RT)

cDNAs were synthesised by diluting 1 μg of total RNA to 7.7 μl with sterilised distilled water, heating to 70°C for 5 min and placing on ice 2 min prior to the addition of reaction mixture containing 1xRT buffer (10 mM Tris pH 9.0, 50 mM KCl, 0.1% Triton X-100), 1 mM dNTPs, 5 mM $MgCl_2$, 20 U RNasin, 20 U AMV reverse transcriptase and 50 μg/ml oligo$(dT)_{15}$ in a volume of 12.3 μl. Following brief centrifugation, the reactions were incubated at 42°C for 45 min and then at 95°C for 3 min. The cDNAs were then cooled on ice before addition of 1 mM EDTA (20 μl) and stored at −70°C without further treatment.

2.4. Polymerase chain reaction (PCR)

PCR amplification was carried out on cDNA equivalent to 100 ng of starting RNA, using separately oligonucleotide primers specific for mouse leptin (forward 5′ GATGACACCAAAACCCTCATCAAG 3′, and reverse 5′ GCCACCACCTCTGTGGAGTAG 3′) and β-actin (forward 5′ ATCCTGCGTCTGGACCTGGCTG 3′, and reverse 5′ CCTGCTTGCTGATCCACATCTGCTG 3′). The actin reverse primer was labelled prior to PCR by mixing 625 ng oligonucleotide, 1× One-Phor-All Plus buffer (10 mM Tris-acetate pH 7.5, 10 mM $Mg(OAc)_2$, 50 mM KOAc), 100 μCi [γ-^{33}P]-ATP (2000 Ci/mmol) and 28.5 U T4 polynucleotide kinase in a final volume of 50 μl. This reaction was incubated at 37°C for 30 min and then at 95°C for 5 min. End-labelled primer was stored at −20°C. PCR mixes contained cDNA, 1 U of Taq polymerase, 1×PCR buffer (20 mM Tris-HCl pH 8.4, 50 mM KCl), 200 μM dNTPs, 2 mM $Mg(OAc)_2$ (leptin) or 1 mM $Mg(OAc)_2$ (actin), 25 ng (leptin) or 12.5 ng (actin) of forward primer and 25 ng of reverse primer (leptin) or 12.5 ng end-labelled reverse primer (actin) in a volume of 10 μl. For each set of tissues (eg. all samples of WAT), a single leptin or actin reaction mix containing all components except the cDNA was prepared for the entire PCR experiment and aliquotted to minimise variation between samples. Each PCR experiment included a negative control consisting of an RT reaction containing no added RNA. PCR was carried out in an FTS-1 capillary thermal sequencer (Corbett Research, Lidcombe, N.S.W). Following initial denaturation of samples by heating at 95°C for 2 min, each cycle of amplification consisted of a stage of denaturation (95°C, 30 s), annealing (56°C for leptin or 64°C for actin, 30 s) and an extension stage (72°C, 30 s). A final extension stage was 72°C for 3 min.

2.5. Detection and quantitation of PCR products

PCR products corresponding to RNA samples from treated and control mice within a given experiment were electrophoresed on the same 1.3% agarose gel and transferred onto a Hybond N^+ nylon membrane by Southern blotting in 0.4 M NaOH/1 M NaCl. Membranes were rinsed for 5 min in 0.5 M Tris-HCl (pH 7.5)/1 M NaCl, then in 2×SSC (0.3 M NaCl/30 mM sodium citrate). Following transfer of [^{33}P]-labelled actin PCR products, nylon membranes were dried at room temperature for 30 min, then apposed directly to phosphorimager (PI) plates. The identity of the unlabelled leptin PCR products was verified by hybridisation to an independent probe (5′ GGCTTGGACTTCATTCCTGGGCT 3′) end-labelled in a 20 μl reaction mix containing 50 ng oligonucleotide, 50 μCi [γ-^{33}P]ATP, 1×One-Phor-All Plus buffer and 9.5 U T4 polynucleotide kinase. The reaction was incubated at 37°C for 30 min, then at 95°C for 5 min before being diluted to 60 μl with sterilised distilled water. The labelled probe was separated from unincorporated nucleotide by centrifugation through Chroma-spin 10 columns (Clontech) according to the manufacturer's instructions. Leptin PCR products were fixed to nylon membranes by exposure to u.v.-light for 2 min before pre-hybridisation at 42°C for 4 h in a buffer containing 5×SSC, 0.5% SDS, 100 μg/ml salmon sperm DNA (pre-heated to 95°C for 5 min), 5×Denhardts solution (1 mg/ml ficoll type 400, 1 mg/ml polyvinylpyrrolidone, 1 mg/ml bovine serum albumin) and 0.1 mM ATP. After addition of the end-labelled oligonucleotide probe (equivalent to 25 ng), hybridisation was performed at 42°C for 16 h. The filters were washed in 2×SSC/0.1% SDS for 30 min at 30°C, then for 5 min at 37°C. Radioactivity was detected with a Molecular Dynamics Phosphorimager (SI) after exposure to imaging plates.

2.6. Statistical analysis

Leptin mRNA levels were corrected for any differences in input RNA by dividing the number of leptin PI units by the number of actin PI units for each cDNA. Results are expressed as mean ± S.E.M. For Southern blot data the mean value for control mice is expressed as 100% (unless otherwise stated). The statistical significance of differences between groups was assessed using a two-tailed Student's unpaired *t*-test. Probability (*P*) values equal to or less than 0.05 were considered significant. To correct for heterogeneous variance (assessed by the *F*-test), Welch's correction was applied.

2.7. Drugs and reagents

Heparin (CSL, Parkville, Vic., Australia); Reserpine, (S)-(−)-propranolol hydrochloride, SR-59230A (gift from Sanofi Recherche, Italy); TRIZOL reagent, oligo$(dT)_{15}$, oligonucleotide primers, Taq polymerase, 10×PCR buffer, salmon sperm DNA (Gibco BRL, Gaithersburg, USA); RNasin, 10×RT buffer, AMV reverse transcriptase (Promega, Annandale, N.S.W., Australia); dNTPs (100 mM each nucleotide), 100 bp DNA ladder, One-Phor-All Plus buffer, T4 polynucleotide kinase (Pharmacia Biotech, Sydney, N.S.W., Australia); [γ-^{33}P]ATP (2000 Ci/mmol: Bresatec, Adelaide, S.A., Australia).

3. Results

3.1. Determination of leptin mRNA levels in mouse tissues by semi-quantitative PCR

The RT-PCR technique used to compare leptin mRNA levels in mouse tissues was validated as described by Evans et al. [20]. PCR was carried out using unlabelled primers and following electrophoresis, PCR products were hybridised with an independent leptin probe (see Fig. 1). Products detected in BAT and WAT were consistent with the expected size of 353 bp. As we used intron-spanning primers, any product derived from contaminating genomic DNA would be 2.1 kb. By doing PCR at different cycle numbers [18,20,22] on WAT and BAT cDNAs equivalent to a set amount of starting total RNA (100 ng), we found that the level of leptin mRNA in BAT is only 2.7% of that in WAT (S.E.M. 0.5%, $n = 4$). Because of this large difference in abundance, we used 22 cycles for WAT and 28 cycles for BAT in all subsequent experiments. For RT-PCR on 100 ng RNA, these cycle numbers were within the range where a plot of $\log_{10}$ PCR product versus PCR cycles was linear [20]. We also verified that at 22 cycles, 100 ng of WAT RNA was within the linear range of a plot of leptin PCR product versus amount of input RNA [20].

3.2. Effect of sympathetic blockade on leptin mRNA and on circulating leptin

Treatment with reserpine produced the expected symptoms such as ptosis and diarrhoea [21]. Fig. 1 displays a Southern blot of leptin and actin PCR products corresponding to WAT and BAT of control mice and of mice treated with reserpine. Fig. 2 shows that reserpine treatment did not significantly alter leptin mRNA abundance in WAT ($P = 0.14$) but increased the level of leptin mRNA in BAT by 3.7-fold ($P = 0.002$). Despite this large increase in leptin mRNA in BAT, reserpine treatment did not significantly affect circulating leptin levels ($P = 0.37$).

3.3. Effect of cold-exposure in the absence and presence of β-AR antagonists

The effect of stimulating the SNS was tested by exposing mice to 4°C for 4 h. Levels of leptin mRNA in BAT decreased

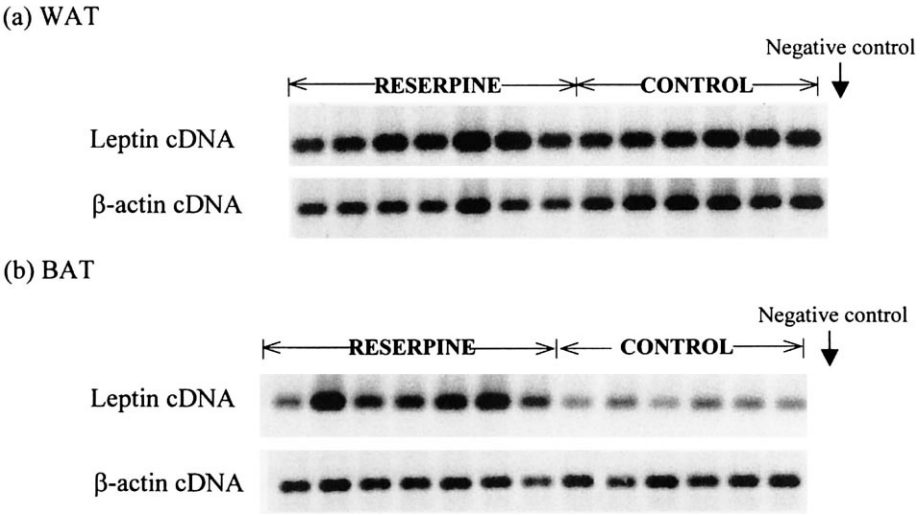

Fig. 1. Southern blots of leptin and actin PCR products in C57Bl/6J mice treated with reserpine ($n=6$) or vehicle ($n=6$). Treated mice received 2 mg/kg reserpine i.p. at time 0, with a second dose of 2 mg/kg i.p. at 20 h. The mice were killed 24 h after the second injection. The figure shows sets of PCR products from tissues of individual mice, a: WAT, b: BAT.

to 12% of those in control mice maintained at room temperature ($P=0.008$, Fig. 3). In WAT there was a smaller decrease to 44% ($P=0.006$) of the level in control mice. Serum leptin in the cold-exposed mice was reduced to 70% ($P=0.02$). To assess the role of β_3-ARs versus classical β_1- and β_2-ARs in mediating the reduction in leptin mRNA, we treated mice 15 min prior to cold-exposure with either propranolol (a β_1- and β_2-AR antagonist), SR 59230A (a selective β_3-AR antagonist), or a combination of both drugs. A second group of mice were treated with the antagonists but left at room temperature for 4 h. As shown in Fig. 3, both propranolol and SR 59230A produced substantial increases in BAT leptin mRNA, to 218% ($P=0.015$) and 306% ($P=0.018$) respectively, even when mice were maintained at room temperature. The combination of propranolol and SR 59230A had an additive effect, increasing leptin mRNA to 495% of that in vehicle-treated mice ($P=0.02$). Different effects were obtained when the mice were cold-exposed following antagonist treatment. Under these conditions of high SNS activity, blockade by either antagonist alone was not sufficient to prevent the large decrease in leptin mRNA (Fig. 3). In the presence of both propranolol and SR 59230A on the other hand, cold-exposed mice showed no significant reduction in BAT leptin mRNA ($P=0.68$).

In the WAT of mice held at room temperature, leptin mRNA levels were not affected by treatment with propranolol or SR 59230A alone, but were increased to 176% in mice treated with the combination of antagonists compared to vehicle ($P=0.02$, Fig. 3). In cold-exposed mice, treatment with propranolol or SR 59230A alone produced a slight but non-significant increase in leptin mRNA relative to cold-exposed mice treated with vehicle ($P=0.28$ and 0.057 respectively). Again the combination of propranolol and SR 59230A prevented the cold-induced decrease in leptin mRNA ($P=0.57$). The results seen in WAT are reflected in serum leptin levels. At room temperature there was no increase due to treatment

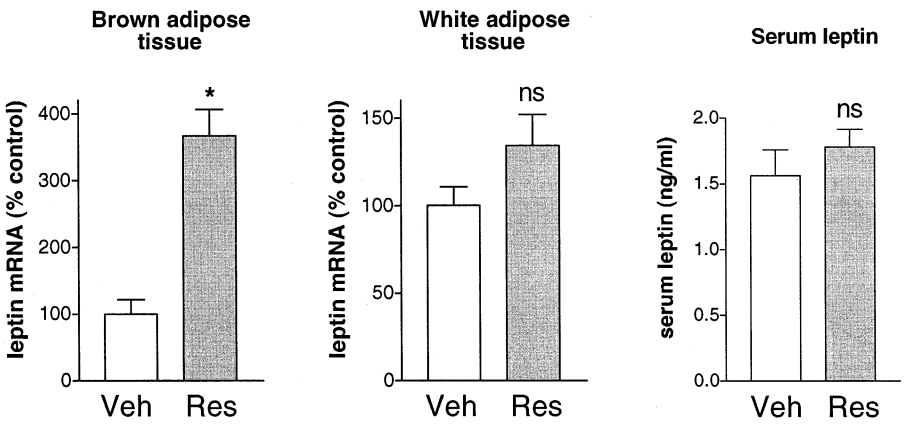

Fig. 2. Relative levels of leptin mRNA and serum leptin in C57Bl/6J mice treated with reserpine or vehicle (as described in Fig. 1). PCR experiments using either leptin or actin primers were carried out separately. Cycle numbers for amplification of leptin cDNA were 28 for BAT and 22 for WAT. Actin PCR products were measured after 16 cycles for both tissues. PCR products were quantified by phosphorimaging and values for leptin product from each individual sample corrected for the signal obtained for the corresponding actin product. Each value was then converted to a percentage of the mean from the vehicle treated animals. Serum leptin was measured using a leptin radioimmunoassay kit. Bars show mean ± S.E.M. ($n=6$–7 separate animals): $^*P<0.05$, no significant difference.

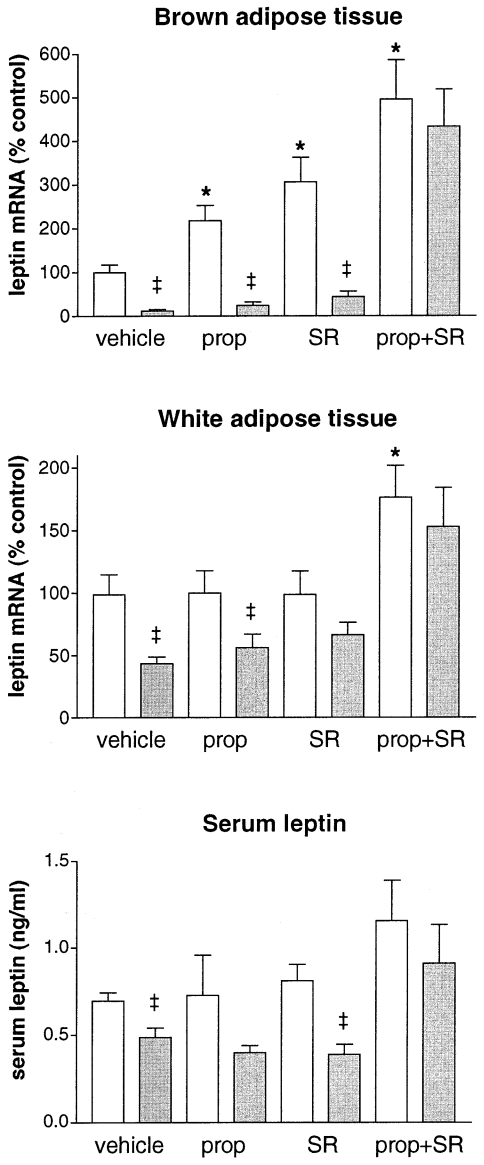

Fig. 3. Relative levels of leptin mRNA and serum leptin in C57Bl/6J mice. Animals were injected i.p. with vehicle, propranolol (1.5 mg/kg), SR 59230A (1.5 mg/kg) or a combination of both antagonists at the same doses. After 15 min at 22°C, the mice were left at 22°C (open bars) or placed at 4°C (filled bars) for 4 h. PCR experiments using either leptin or actin primers were carried out separately. Cycle numbers for amplification of leptin cDNA were 28 for BAT and 22 for WAT. Actin PCR products were measured after 16 cycles for both tissues. PCR products were quantified by phosphorimaging and values for leptin product from each individual sample corrected for the signal obtained for the corresponding actin product. Each value was then converted to a percentage of the mean from the vehicle treated animals left at 22°C. Serum leptin was measured using a leptin radioimmunoassay kit. Bars show mean ± S.E.M. ($n = 5$–10 separate animals): *$P < 0.05$, effect of β-antagonists versus saline in mice held at 22°C: ‡$P < 0.05$, mice at 4°C versus 22°C for a given drug treatment.

with propranolol or SR 59230A, whereas the combination of antagonists appeared to increase serum leptin (non-significant, $P = 0.109$). Cold-exposure for 4 h reduced serum leptin in mice treated with any of vehicle, propranolol or SR 59230A. When mice were pre-treated with propranolol plus SR 59230A, cold-exposure no longer produced a significant decrease in serum leptin ($P = 0.29$).

4. Discussion

This study provides evidence that outflow from the SNS stimulates both β_3-ARs and the classical β_1/β_2-ARs. This happens to the greatest extent in BAT, which is highly innervated by the SNS [22]. Our results indicate that even at room temperature there is enough sympathetic tone to suppress expression of BAT leptin mRNA (Fig. 3). This suppression can be alleviated by depleting sympathetic nerve terminals of catecholamines with reserpine (Figs. 1 and 2) [21], or by blockade of β-ARs. The effects of SR 59230A and propranolol at room temperature demonstrate that β_3-ARs as well as classical β_1/β_2-ARs mediate the response to basal sympathetic tone. Our finding that the effect of SR 59230A and propranolol in combination is additive compared to the effect of either single antagonist could be interpreted in two ways. One is that the responses mediated by β_3-ARs occur via a different signalling pathway to those mediated by β_1/β_2-ARs. This seems unlikely as there is convincing evidence that all β-ARs are coupled primarily to G_s in BAT [23,24]. Increases in cAMP modulate leptin gene expression, as cAMP analogues and agents which activate adenylate cyclase have been shown to reduce leptin mRNA in vitro [5,14,16]. The amount of cAMP generated due to basal stimulation of all three β-ARs clearly causes partial suppression of leptin gene expression in BAT. When the amount of cAMP is reduced due to receptor blockade, there may be incremental increases in gene expression, reaching a peak when all β-ARs are blocked.

When the SNS is stimulated due to cold-exposure, the large increase in sympathetic outflow would result in substantial increases in cAMP generation [25]. Under these conditions blockade of either β_3-ARs or β_1/β_2-ARs alone is insufficient to reverse the cAMP-dependent suppression of leptin gene expression. It is only when all three β-ARs are blocked that the cold-induced increase in cAMP is prevented and leptin mRNA remains at the same level as in the corresponding animals held at room temperature.

Our finding that propranolol plus SR 59230A prevents the decrease in leptin mRNA also suggests that no other receptors contribute substantially to the response to cold-exposure in BAT. This is interesting given that α-ARs are possible candidates for modulation of leptin synthesis. α_1-ARs are present in BAT, and activation of these receptors is coupled to a stimulation of oxygen consumption [26]. α_{1A}-ARs in the BAT of Sprague-Dawley rats have been shown to be up-regulated by cold-exposure (4°C, 4 days) and infusion of the β_3-AR agonist CL 316243, both of which down-regulate leptin synthesis [27]. Furthermore, activation of α_1-ARs potentiates the thermogenic response by synergistically activating adenylate cyclase thereby increasing cAMP [28]. Further studies using α_{1A}-AR antagonists would be worthwhile to investigate whether these receptors do play any role in regulating BAT leptin synthesis. Another receptor which appears to play a role in BAT oxygen consumption is the putative β_4-AR [29], which mediates a maximal response to the agonist CGP 12177 in both wild-type and β_3-AR knock-out mice. However CGP 12177 has been shown to have β_1-AR agonist properties in cells expressing this receptor [30], so that the existence of the

β_4-AR and its possible contribution to the regulation of leptin synthesis remain unconfirmed.

Although leptin mRNA is abundant in cultured brown adipocytes [5], leptin protein is undetectable in UCP-1 expressing cells in vivo [4]. Instead leptin is present in white adipocytes found within BAT depots of lean mice. We addressed directly the relative abundance of leptin mRNA in BAT and WAT, finding that for a fixed amount of total RNA the level in BAT is only 2.7% of that found in WAT. Due to the large size of white adipocytes, however, the yield of total RNA per wet weight of tissue is 7-fold higher for BAT (865 ± 104 μg/g, $n = 12$) than for WAT (123 ± 9 μg/g, $n = 12$). Taking this into account the level of leptin mRNA for a given weight of BAT would be 19% of that in WAT. Our study provides evidence that even when BAT leptin mRNA is increased 5-fold (by treatment of mice at room temperature with propranolol plus SR 59230A), there is not a corresponding large increase in serum leptin levels. The data shown in Fig. 2 and 3 indicate that serum leptin essentially reflects expression in WAT. This is consistent with the relatively low body content of BAT compared to WAT [17].

Activation of the SNS by acute cold-exposure reduced leptin synthesis by 10-fold in BAT but only by 2-fold in WAT. This effect is much less marked than that seen by Trayhurn et al. [9], who demonstrated that even after 2 h of cold-exposure of mice, leptin mRNA was undetectable in WAT. The difference between the two studies may reflect the conditions of cold-exposure, or a difference between the C57BL/6 strain and the 'Aston' variety used by Trayhurn and coworkers. Moinat et al. [10] found that exposure of male Sprague-Dawley rats to 6°C for 24 h had no effect on leptin mRNA in WAT although leptin expression was reduced significantly in BAT. Again the different animal and the time and conditions of cold-exposure may have influenced the results obtained.

In mice, the observed reduction in WAT leptin mRNA with cold-exposure may be due to activation of the SNS or increased circulating catecholamines. Our data do not distinguish clearly between these two possibilities. It had previously been supposed that WAT is poorly innervated by the SNS, with nerve terminals localised mainly in the proximity of blood vessels [31]. However, 4 h cold-exposure of rats has been shown to increase noradrenaline turnover by 3-fold in WAT compared to 5-fold in BAT [32], indicating significant sympathetic activity in WAT. Similarly, fasting appears to selectively activate sympathetic stimulation to WAT whilst decreasing activity in BAT [33]. In our study, sympathetic blockade due to reserpine treatment caused a small but non-significant increase in WAT leptin mRNA (Fig. 2). In mice held at room temperature, separate blockade of β_1/β_2-ARs or β_3-ARs using propranolol or SR 59230A produced no change, but the combination of antagonists caused a 1.8-fold increase in leptin mRNA (Fig. 3). It is interesting to compare these results with those of Rayner and coworkers [11], who found a 3-fold increase in leptin mRNA 10 h after treatment of mice with αMPT. This agent inhibits noradrenaline synthesis in sympathetic nerve terminals, but may also cause depletion of circulating adrenaline [11]. Taken together, the data are consistent with a chronic suppression of WAT leptin expression at room temperature caused partly by basal sympathetic tone and partly by adrenaline. Under conditions of acute cold-exposure, there may be increases in circulating adrenaline as well as elevated sympathetic activity. Whichever mechanism operates in cold-exposed mice, blockade of all three β-ARs is clearly necessary to prevent the decrease in WAT leptin mRNA.

In conclusion, we have shown that agents which modulate sympathetic activity and responsiveness alter leptin gene expression primarily in BAT, and to a lesser extent in WAT. This directly confirms the hypothesis that the SNS regulates leptin synthesis in tissues that receive sympathetic innervation. Importantly, responses to basal and stimulated sympathetic activity in BAT and WAT are mediated by both β_3-ARs and classical β_1/β_2-ARs. WAT is the primary source of circulating leptin, despite large increases in BAT leptin mRNA following reserpine or β-AR antagonist treatment.

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