FEBS Letters 455 (1999) 170–174 FEBS 22325

Leptin treatment increases suppressors of cytokine signaling in central and peripheral tissues

Valur Emilsson[a,*], Jonathan R.S. Arch[b], Rolf P. de Groot[c], Carolyn A. Lister[b], Michael A. Cawthorne[a]

[a] *Clore Laboratory, University of Buckingham, Hunter Street, Buckingham, UK*
[b] *SmithKline Beecham Pharmaceuticals, Harlow, Essex, UK*
[c] *Department of Pulmonary Diseases, University Hospital Utrecht, Utrecht, The Netherlands*

Received 29 April 1999; received in revised form 21 June 1999

Abstract Leptin concentrations are elevated in the majority of obese individuals raising the possibility that leptin resistance contributes to their obesity. Peripheral leptin administration for 48 h caused a several-fold increase in mRNA encoding the suppressors of cytokine signaling SOCS-3 and CIS in hypothalamus and peripheral tissues. Paradoxically, CIS and SOCS-3 mRNAs are also elevated in the leptin-deficient *ob/ob* mouse. Forced expression of CIS in insulinoma cells prevented transactivation mediated by leptin. Thus tissues continuously exposed to leptin and/or other factors associated with obesity accumulate excessive amounts of SOCS-3 and CIS which could provide a potential mechanism for leptin resistance.

Key words: Leptin; Signal transducer and activator of transcription; Suppressor of cytokine signaling; Cytokine-inducible SH2-containing protein; Leptin resistance

1. Introduction

Leptin and leptin receptor (OB-R) deficiencies result in profound obesity and endocrine dysfunction [1–3]. However, mutations in the leptin system are rarely the cause of obesity in humans. Instead, a parallel elevation of leptin levels with increased obesity is observed in most rodent obesity models and obese humans, raising the possibility that resistance to the action of leptin contributes to their obesity [4,5]. Resistance to exogenous leptin has been established in diet-induced rodent obesity which is a model that resembles human obesity [6,7]. Although the causes and mechanism(s) of leptin resistance have not yet been defined, high leptin concentrations could have detrimental effects on leptin receptor number or on a signaling molecule.

The OB-R is closely related to the class I cytokine receptor family, which includes for instance the granulocyte colony-stimulating factor and gp130, the common signal transducing chain of the receptors for interleukin 6 and leukemia inhibitory factor [1,2]. These receptors contain sequence motifs that are required for interaction with the Janus kinases (JAKs) and signal transducers and activators of transcription (STATs). STAT proteins are recruited to the activated receptor-JAK complex through Src-homology domains (SH2, SH3) and are activated by tyrosine phosphorylation. In a number of in vitro and in vivo studies, leptin has been shown to activate the JAK/STAT signal transduction cascade [8–10]. Thus, leptin activates a STAT-3-dependent satiety mechanism in the mouse hypothalamus [8], STAT-3 in rat isolated pancreatic islets [9] and STAT-5 in small intestine epithelium [10]. Leptin-mediated STAT-3 and STAT-5 signaling in native islets and small intestine has been corroborated by nuclear activation of STAT-3 in clonal insulin-secreting cells and activation of STAT-5 in CACO-2 cells which are a human model of small intestine epithelium [9,10]. Finally, leptin has been found to modulate insulin action in hepatic cells by affecting activities of the insulin receptor substrate 1 (IRS-1) and IRS-1-associated phosphatidylinositol 3-kinase [11].

Some of the immediate-early genes induced by the STAT factors encode the suppressors of cytokine signaling (SOCS) and cytokine-inducible SH2-containing protein (CIS) [12,13], which regulate the intensity and duration of cytokine signal transduction. The SOCS and CIS contain SH2 domains that can mask phosphotyrosine residues on the cytokine receptor and/or the catalytic region of JAK thus preventing activation of the STAT proteins [12,13]. Acute (2 h) leptin treatment has been shown to stimulate expression of SOCS-3 in hypothalamus of the *ob/ob* mouse and forced expression of SOCS-3 prevented leptin-mediated signal transduction [14]. In the present study we determined the effect of leptin treatment for 48 h on the expression of key molecules involved in the leptin system of lean and obese (*ob/ob*) mice. Leptin had no effect on the OB-R, STAT-3, STAT-5, SOCS-1 and SOCS-2 mRNA levels. In contrast, leptin treatment induced SOCS-3 and CIS mRNA by two to nine-fold in many target tissues for leptin. Forced expression of CIS in clonal insulin-secreting cells, prevented transcriptional activation of STAT-3-dependent reporter constructs by leptin. Thus tissues exposed to relatively high levels of leptin, as found in states of obesity, accumulate excessive amounts of the suppressors of leptin signaling, SOCS-3 and CIS, which could be a potential mechanism for leptin resistance.

2. Materials and methods

2.1. Synthesis, purification and characterisation of leptin

Recombinant murine leptin was produced and purified in *Escherichia coli* as described previously [15,16], except that the gene was cloned without N-terminal tags and expressed from a T7 promoter in the vector pBroc413. The recombinant leptin material was ascertained to be endotoxin-free by the Limulus amoebocyte lysate test

*Corresponding author. Fax: (44) (1280) 820135.
E-mail: valur.emilsson@buckingham.ac.uk

Abbreviations: OB-R, leptin receptor; STAT, signal transducers and activators of transcription; SOCS, suppressors of cytokine signaling; CIS, cytokine-inducible SH2-containing protein; i.p., intraperitoneal; RT-PCR, reverse transcription-polymerase chain reaction; GAS, interferon-γ-activated sequence; IRE, interferon-γ/interleukin-6 response element

PII: S0014-5793(99)00874-1

[15,16]. The purity was ›95% and the material has been shown previously to inhibit food intake when given either centrally or peripherally [15].

2.2. Animals and treatment

Lean (+/?) and obese (*ob/ob*) mice, aged 5–6 weeks, were obtained from Harlan Olac (UK) and housed in cages with metal grid floors on a 12:12 h light-dark cycle and given unrestricted access to water and powdered RM1 maintenance diet. Mice were then injected intraperitoneally (i.p.) twice a day (08.00 and 17.00 h) with 1.5 mg/kg recombinant murine leptin or saline vehicle for 2 days. Food intake (correcting for spillage) and body weight were measured daily at 08.00 h. Mice were killed on the morning following the fourth dose of leptin or vehicle in the fed state by cervical dislocation prior to the removal of the following tissues: whole brain, hypothalamus, liver, white adipose tissue (WAT) and small intestine.

2.3. Messenger RNA analysis

Total RNA was isolated using Tri Reagent (Amersham) and isopropanol precipitation. The integrity and loading of the RNA was studied by detection scanning of ribosomal RNA bands (28S and 18S) in agarose gels before and after treatment with RNase-free DNase I (Gibco/BRL). Single stranded cDNA synthesis was performed from approximately 2 μg of total RNA using oligo$(dT)_{15-18}$ (Invitrogen) in a first-strand synthesis kit (Pharmacia Biotech). mRNA expression was determined by PCR using the following primer sequences: 5′-ATGGTCCTCTGCGTACAG-3′ and 5′-TGACAAGCAGTT AGAGTC-3′ to amplify a 440 bp DNA fragment of SOCS-1 (GenBank U88325), 5′-ATGGTA GCACGCAACCAGGTG-3′ and 5′-CTCCAGCAGCTCGAAAAGGCA-3′ to amplify a 460 bp DNA fragment of SOCS-2 (GenBank U88327), 5′-CCATGACCCTGCGGTGCCTGG-3′ and 5′-GGAATTTATATTCTTCCAAGT-3′ to amplify a 593 bp DNA fragment of SOCS-3 (GenBank U88328), 5′-GGAGTGGTGGCTCCTGGCTCT-3′ and 5′-GGTAATTGCATGG CTGCTGCA-3′ to amplify a 630 bp DNA fragment of CIS (GenBank D31943), 5′-GCTG GATGAAAGGGGACTTG-3′ and 5′-GTGACTTCCATACGCAAACC-3′ to amplify a 348 bp fragment of OB-R (common to all isoforms) (GenBank U53144), 5′-CAGAAAGTGTCCTACAAGGGCG-3′ and 5′-CGTTGTTAGACTCCTCCATGTTC-3′ to amplify a 239 bp fragment of STAT-3 (α and the β isoforms) (GenBank U06922), 5′-CATCACGGACATCATCTCAGC-3′ and 5′-GACATGTTTCTGAAGTGGGCG-3′ to amplify a 302 bp fragment common to STAT-5a and STAT-5b (GenBank Z48538) and 5′-CTCTTTAATGTCACGCACGAT-3′ and 5′-AGTGCTGTGGGTGTAGGTACT-3 were used to amplify 534 bp of β-actin (GenBank J00691). PCR products were then cloned directly into a PCR-TRAP cloning system (GeneHunter Corp., USA) and the identity of PCR products confirmed by sequencing using Thermo-Sequenase terminator cycle sequencing kit (Amersham Life Sciences, UK). Quantitation of mRNA expression was performed by quantitative RT-PCR and ethidium bromide staining as described previously [10,17]. Thus cDNA samples were diluted serially then split equally and used either for PCR amplification of the test cDNA and the housekeeping β-actin cDNA at a fixed number of PCR cycles (35 cycles for a test cDNA and 30 cycles for β-actin). Bands in agarose gels were quantitated by scanning laser densitometry and changes in the intensity of test cDNA then normalised to the intensity of the β-actin cDNA, in order to provide information on the relative changes of tissue gene expression. Results are expressed as mean ± S.E.M. and statistical significance assessed using Student's unpaired *t*-test.

2.4. Cell culture and transient transfection

RINm5F cells were routinely cultured as described previously [18]. The reporter constructs used were chloramphenicol acetyl transferase (CAT) containing four copies of the human FcγRI interferon γ activated sequence (GAS) 5′-AGCTTGAGATGTATTTCCCAGAAAAGA-3′, or four copies of the human ICAM-1 interferon response element (IRE), 5′-AGCTTAGTTTCCGGGAAAGCAC-3′, or four copies of the β-casein STAT binding site (β-CAS), 5′-AGCTTAGATTTCTAGGAATTCAAATCA-3′. STAT binding sites were cloned into the thymidine kinase (TK)-CAT vector pBLCAT2 (pBL; pBluescript) using a method described previously [19]. pSG5-mCIS contains the mouse CIS cDNA cloned into the pSG5 eucaryotic expression vector (Stratagene). For transfection experiments, cells were split 1:3 and 24 h later transfected with 10 μg supercoiled plasmid DNA by the DEAE-dextran technique. For co-transfection experiments, 12–18 μg of plasmid DNA was used (10 μg reporter and 2–8 μg pSG5-CIS). Transfected cells were incubated for 6 h at 37°C. Cells were then washed twice with PBS, and fresh medium was added. 24 h later cells were serum-starved for 16 h, after which the cells were stimulated for 12 h with leptin (100 nM) or vehicle and subsequently harvested for CAT assay.

3. Results and discussion

Leptin, produced predominantly by the adipose tissue, provides a negative feedback to the the hypothalamus, reducing food intake and increasing energy expenditure [1]. Elevation of leptin levels with increased obesity is observed in most rodent obesity models and obese humans, raising the possibility that resistance to the action of leptin contributes to their condition [4,5]. For instance, leptin expression and secretion is increased by 10–20-fold in adipose tissue of obese mice relative to the lean littermates [20,21]. In the present study, we determined the effect of leptin treatment for 48 h on the expression of key molecules involved in the leptin system as well as the effect on food intake and body weight of lean and obese (*ob/ob*) mice. The dose of leptin used, 1.5 mg/kg i.p. twice daily, is a sub-maximal leptin concentration within the dose-response range originally reported to show leptin-mediated inhibition of food intake and activation of the JAK/STAT signaling pathway [10,22,23]. Leptin reduced food intake in lean mice over the 2 day treatment period by 26% (8.64 ± 0.22 in control vs 7.23 ± 0.41 in leptin-treated; $n = 9$, $P < 0.05$) and in obese mice by 35% (8.41 ± 0.53 in control vs 5.50 ± 0.34 in leptin-treated $n = 9$, $P < 0.001$). Following leptin treatment for 48 h, body weight was reduced in lean mice (weight change: $+1.37 \pm 0.60$ g in control vs -3.24 ± 0.40 g in leptin-treated;

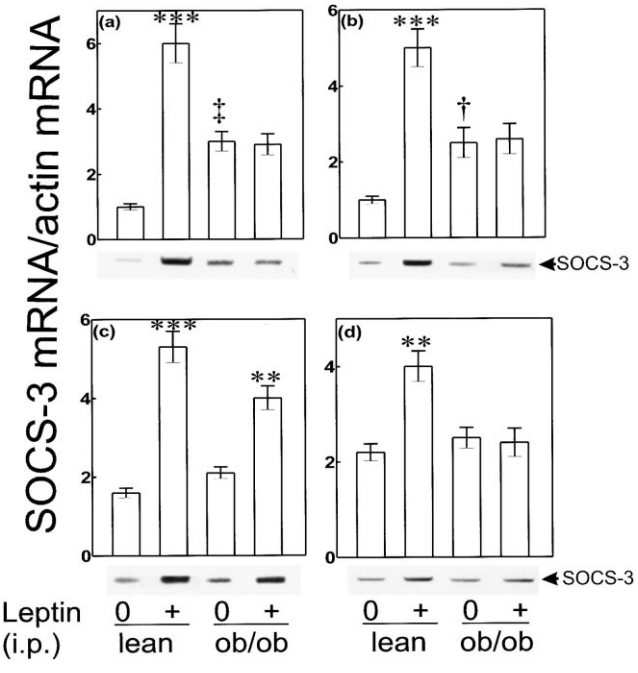

Fig. 1. The effect of 48 h leptin treatment (2×1.5 mg/kg i.p. twice per day) on SOCS-3 mRNA levels in (a)whole brain, (b) hypothalamus, (c) liver and (d) small intestine. Results are expressed as mean ± S.E.M. and statistical significance assessed using Student's unpaired *t*-test. $^{**}P < 0.001$, $^{***}P < 0.0002$ vs 0 leptin. $^{\dagger}P < 0.004$, $^{\ddagger}P < 0.001$ vs lean mice. PCR products are displayed together with the quantitation of SOCS-3 mRNA relative to β-actin.

Table 1
The effect of sub-chronic leptin treatment on OB-R, STAT-3, STAT-5 and SOCS-2 mRNA levels in lean (+/?) and obese (*ob/ob*) mice

mRNA	Whole brain				Hypothalamus				Liver				Small intestine				WAT			
	lean		ob/ob		lean		ob/ob		lean		ob/ob		lean		ob/ob		lean		ob/ob	
	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+
OB-R	1.9	1.7	2.5	2.2	0.9	0.8	1.8	1.6	1.8	1.5	1.8	2.0	2.4	2.6	2.7	2.4	0.4	0.5	0.4	0.6
STAT-3	0.4	0.5	0.5	0.5	0.7	0.8	0.6	0.7	0.8	0.6	0.7	0.6	0.4	0.3	0.4	0.4	0.5	0.5	0.6	0.5
STAT-5	0.7	0.6	0.6	0.6	0.9	1.2	1.1	1.0	1.4	1.2	1.6	1.5	1.6	1.8	1.7	1.6	0.7	0.6	0.7	0.8
SOCS-2	1.5	1.4	1.7	1.5	2.3	2.5	2.1	2.5	1.3	1.2	1.1	1.2	2.5	2.7	2.2	2.3	2.5	2.7	2.2	2.4

mRNA expression is relative to β-actin (arbitrary units) and results are expressed as mean of four independent experiments with S.E.M. of ±10–18%. Statistical significance was assessed using Student's unpaired *t*-test. $^{\dagger}P<0.04$ and $^{\ddagger}P<0.001$ vs lean mice. Mice were treated with recombinant leptin (+) twice daily (2×1.5 mg/kg) for 2 days or with PBS without leptin (−).

$n=9$, $P<0.001$) and in the obese mice (weight change: +0.84±0.37 g in control vs −3.42±0.70 g in leptin-treated; $n=9$, $P<0.001$).

Leptin mediates its diverse biological effects by activating the JAK/STAT pathway through the OB-Rb receptor isoform in both central and peripheral tissues [8–10]. Using quantitative RT-PCR as described previously [17], we find that the leptin treatment had no effect on the OB-R (common to all OB-R splice variants), STAT-3, STAT-5 and SOCS-2 mRNA levels in whole brain, hypothalamus, small intestine, liver and WAT of the lean or obese (*ob/ob*) mice as summarised in Table 1. SOCS-1 was not detected by RT-PCR in any of these tissues from either treated or untreated animals. OB-R mRNA levels were increased by 1.3-fold ($P<0.04$) in whole brain and by 2-fold ($P<0.001$) in hypothalamus of *ob/ob* mice compared with the lean littermates (Table 1). Western blot analysis using antibodies raised against the N-terminal domain of the OB-R confirmed the results, showing no effect of chronic leptin treatment on OB-R mRNA levels (data not shown).

The SOCS proteins are a family of negative regulators of cytokine signal transduction [13]. Following the 48 h leptin treatment, the SOCS-3 mRNA levels were increased by 6-fold ($P<0.0002$) in whole brain, 4.8-fold ($P<0.0002$) in hypothalamus, 3.3-fold ($P<0.0002$) in liver and 1.8-fold ($P<0.001$) in small intestine of lean mice (Fig. 1). Leptin also increased SOCS-3 mRNA by 1.9-fold ($P<0.001$) in liver of *ob/ob* mice compared with untreated *ob/ob* mice (Fig. 1). Paradoxically, SOCS-3 mRNA levels were increased by 3-fold ($P<0.001$) in whole brain, 2.5-fold ($P<0.004$) in hypothalamus of *ob/ob* mice compared with same tissues of lean mice (Fig. 1).

The cytokine-inducible gene CIS, distantly related to the SOCS proteins, is a target of the JAK/STAT pathway and has been shown to suppress tyrosine-phosphorylation of STAT-5 and STAT-5 mediated transactivation in haematopoietic cells [24]. Following the 48 h leptin treatment in the present study, CIS mRNA levels were increased by 9.3-fold ($P<0.0001$) in whole brain, 5.2-fold ($P<0.0001$) in hypothalamus, 2.3-fold ($P<0.0002$) in liver and 3.9-fold ($P<0.0001$) in small intestine of lean mice (Fig. 2). Furthermore, leptin treatment increased CIS mRNA levels by 2.1-fold ($P<0.004$) in hypothalamus, 1.7-fold ($P<0.004$) in liver and 1.4-fold ($P<0.01$) in small intestine of *ob/ob* mice compared with untreated *ob/ob* mice (Fig. 2). Similar to SOCS-3 mRNA levels, CIS mRNA levels were paradoxically increased in whole brain by 4.2-fold ($P<0.0002$), 2-fold ($P<0.004$) in hypothalamus and 2.8-fold ($P<0.0002$) in small intestine of *ob/ob* mice compared with same tissues of lean mice (Fig. 2). Leptin treatment had no effect on CIS or SOCS-3 mRNA levels in WAT nor did we detect any differences in CIS or SOCS-3 mRNA levels in WAT of *ob/ob* mice relative to the lean littermates (data not shown).

To date, only SOCS-3 has been shown to be able to prevent leptin-mediated signaling [14]. The finding in the present study that leptin induced CIS mRNA levels, led us to investigate whether CIS was also a negative regulator of leptin signaling in the leptin-responsive cell line RINm5F. Leptin (100 nM) increased transcriptional activation from reporter plasmids containing GAS (from the FcγRI promoter) and IRE (from the human ICAM-1 promoter) STAT binding elements (Fig. 3A). Leptin-induced transcriptional activity was greater with the IRE (which has a high affinity for STAT-3) compared with the GAS construct (which has a high affinity for

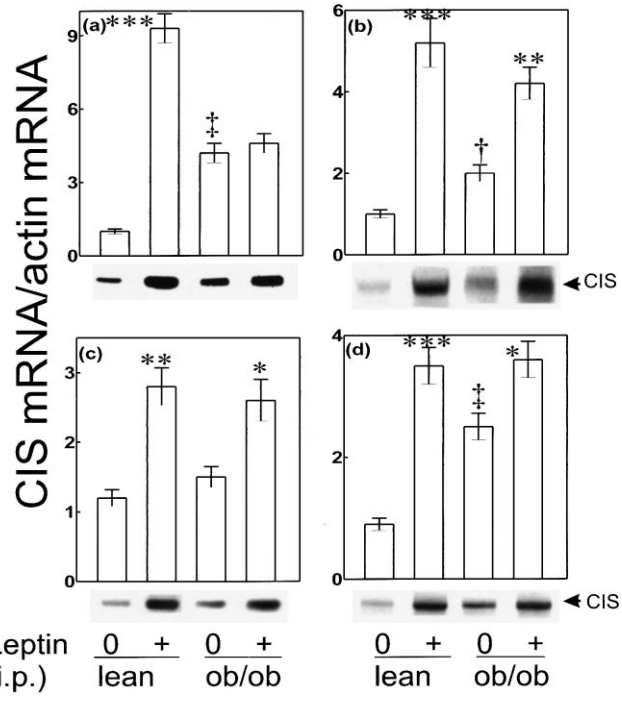

Fig. 2. The effect of 48 h leptin treatment (2×1.5 mg/kg i.p. twice per day) on CIS mRNA levels in (a) whole brain, (b) hypothalamus, (c) liver and (d) small intestine. Results are expressed as mean±S.E.M. and statistical significance assessed using Student's unpaired *t*-test. $^{**}P<0.002$, $^{***}P<0.0002$ vs 0 leptin, $^{\dagger}P<0.004$, $^{\ddagger}P<0.0006$ vs lean mice. PCR products are displayed together with the quantitation of CIS mRNA relative to β-actin.

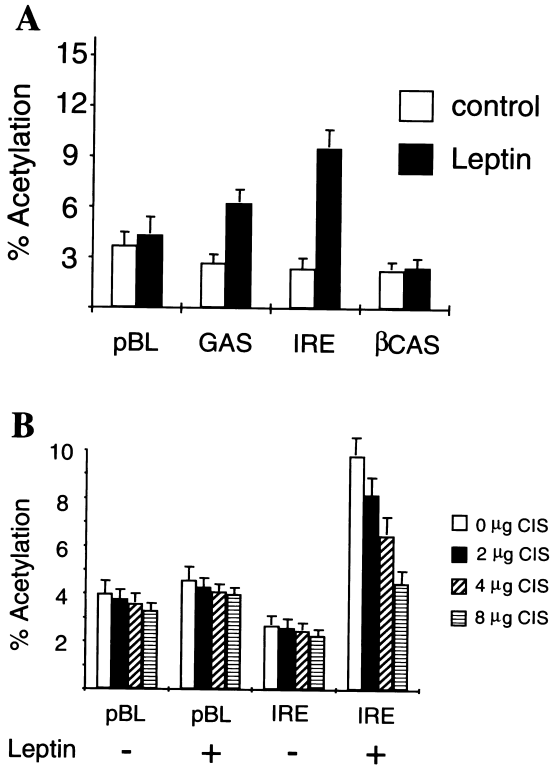

Fig. 3. Transcriptional activation of STAT-dependent reporter constructs by leptin and the effect of forced expression of CIS in rat insulinoma cells. A: RINm5F cells were transiently transfected with STAT-dependent reporter plasmids pBLCAT2 (pBL; pBluescript) as described in Section 2 and indicated below the axis. After a period of serum starvation, the RINm5F cells were exposed to leptin (100 nM) for 12 h and then assessed for induction of CAT activity. No induction was observed from the empty vector pBL (pBLCAT2). B: Forced expression of CIS by the pSG5-CIS eucaryotic expression vector in RINm5F cells, which was cotransfected (2–8 μg pSG5-CIS/transfection) with the IRE reporter plasmid (10 μg), prevented leptin-mediated transactivation.

STAT-1) consistent with STAT-3 activation (Fig. 3A). CIS was then co-transfected at different doses (2–8 μg/transfection) together with the IRE reporter plasmid (fixed at 10 μg) and found to prevent leptin mediated transactivation of the IRE promoter in a dose-dependent manner (see Fig. 3B). Thus in addition to SOCS-3 as described previously [14], leptin induces CIS expression, which can subsequently suppress leptin signaling.

Recently, Bjoerbaek et al. [14] demonstrated that acute (2 h) peripheral leptin administration (1–2 mg/kg, i.p.) induced SOCS-3 mRNA expression in the *ob/ob* mouse hypothalamus, whilst leptin had not effect on the levels of SOCS-1, SOCS-2 or CIS mRNA. Furthermore, forced expression of SOCS-3, but not CIS or SOCS-2, prevented leptin-mediated signal transduction in CHO cells that were cotransfected with the OB-Rb isoform [14]. In the present study, we find that a similar dose of leptin administered for 48 h induced expression of both SOCS-3 and CIS in hypothalamus and peripheral tissues. We also find that forced expression of CIS in clonal β-cells that express the OB-Rb isoform prevented in a dose-dependent fashion leptin-mediated STAT-3 transactivation. It is possible that the difference in the duration of the leptin treatment, 2 h vs 48 h, as well as the difference in the cellular system used to study effects of CIS on leptin signaling, could explain the discrepancies between the present report and that of Bjoerbaek et al. [14].

Cellular responses to cytokines are tightly controlled. An essential step involved in this control is termination of the cytokine signals. Multiple cytokines stimulate transcription of the *SOCS* and *CIS* genes which encode Src-homology (SH2) domains that can mask phosphotyrosine residues on key molecules involved in the JAK/STAT signal transduction. Thus CIS binds to phosphotyrosine residues of the β-chain of interleukin 3 receptor as well as the erythropoietin receptor, preventing activation of the STAT proteins [12]. This results in the termination or dampening of the cytokine signal transduction. Continuous expression of these suppressors could render tissues unresponsive to cytokine action. Conversely, inhibition of STAT activation would result in the reduction of SOCS gene expression and a regain of responsiveness to the particular cytokine. Loss of suppressors by other means, for instance due to mutations in the SOCS and CIS genes, would result in hyperresponsiveness that could contribute to the development of a disease. We have found that 48 h leptin treatment can lead to elevation of the SOCS-3 and CIS mRNA levels in central and peripheral tissues. Moreover, both SOCS-3 and CIS can prevent leptin signal transduction. This suggests that leptin resistance is a consequence of obesity and high leptin levels, although we cannot rule out the possibility that in some individuals it may also be a cause.

We have also shown that SOCS-3 and CIS mRNA levels are paradoxically increased in the hypothalamus of leptin deficient *ob/ob* mice relative to the lean controls. Since *ob/ob* mice are sensitive to the effects of leptin on feeding behaviour, this difference in SOCS-3 and CIS mRNA expression is apparently inconsistent with the proposed role as suppressors of leptin signaling. However there was also a two-fold increase in OB-R in the *ob/ob* hypothalamus and this change could well more than counteract the effects of increases in cytokine suppressors. Further, although leptin had less effects on SOCS-3 and CIS mRNA levels in the *ob/ob* mouse, leptin treatment caused a significant increase in CIS mRNA in hypothalamus and some peripheral tissues of the *ob/ob* mouse suggesting that these molecules have dampened the response to leptin but not turned it off. Our results suggest that some tissues in the *ob/ob* mouse might be less sensitive to leptin compared with same tissues in the lean littermate. In fact, we have recently found that SOCS-3 is increased in the pancreatic islets of the *ob/ob* mouse relative to the lean littermate and this is associated with reduced potency of leptin to inhibit stimulated insulin secretion in *ob/ob* islets (unpublished results). Finally, our results showing increased basal levels of SOCS-3 and CIS in the leptin-deficient *ob/ob* mice suggest that factors other than leptin, that are associated with obesity, can also induce expression of these suppressors. In summary, we have demonstrated that leptin treatment induces levels of the suppressors of leptin signaling, SOCS-3 and CIS, in central and peripheral tissues of both lean and *ob/ob* mice which could be a potential mechanism for leptin resistance.

Acknowledgements: We thank Owen Jenkins, Diane McBay and Gillian Sarginson at SmithKline Beecham for the murine leptin, and Dr Robyn Starr, at the Walter and Eliza Institute for Medical Research Australia, for providing the primer sequences for SOCS and CIS. We are also grateful to Amber Kanu at the Clore Laboratory for technical assistance.

References

[1] Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L. and Friedman, J.M. (1994) Nature 372, 425–432.
[2] Chen, H., Churfat, O., Tartaglia, L.A., Woolf, E.A., Wang, X., Ellis, S.J., Lukey, N.D., Culpepper, J., Moore, K.J., Breitbart, L.F., Duyk, G.M., Tepper, R.L. and Morgenstern, J.P. (1996) Cell 84, 491–495.
[3] Lee, G-H., Proenca, R., Montez, J.M., Carroll, K.M., Darvishzadeh, J.G., Lee, J.I. and Friedman, J.M. (1996) Nature 379, 632–635.
[4] Frederich, R.C., Hamann, A., Anderson, S., Lollman, B., Lowell, B.B. and Flier, J.S. (1995) Nature Med. 1, 1311–1314.
[5] Considine, R.V., Sinha, M.K., Heiman, M.L., Kriauciunas, A., Stephens, T.W., Nyce, M.R., Ohannesian, J.P., Marco, C.C., McKee, L.J. and Bauer, T.L. (1996) New Engl. J. Med. 334, 292–295.
[6] Van Heek, M., Compton, D.S., France, C.F., Tedesco, R.P., Fawzi, A.B., Graziano, M.P., Sybertz, E.J., Strader, C.D. and Davis Jr., H.R. (1997) J. Clin. Invest. 99, 385–390.
[7] Kesterson, R.A., Huszar, D., Lynch, C.A., Simerly, R.B. and Cone, R.D. (1997) Mol. Endocrinol. 11, 630–637.
[8] Vaisse, C., Halaas, J.L., Horvath, C.M., Darnell, J.E., Stoffel, M. and Friedman, J.M. (1996) Nature Genet. 14, 95–97.
[9] Morton, N.M., Emilsson, V., deGroot, R.P., Pallett, A.L. and Cawthorne, M.A. (1998) J. Mol. Endocrinol. 22, 173–184.
[10] Morton, N.M., Emilsson, V., Liu, Y.-L. and Cawthorne, M.A. (1998) J. Biol. Chem. 273, 26194–26201.
[11] Cohen, B., Novick, D. and Rubinstein, M. (1996) Science 15, 1185–1188.
[12] Yoshimura, A., Ohkubo, T., Kiguchi, T., Jenkins, N.A., Gilbert, D.J., Copeland, N.G., Hara, T. and Miyajima, A. (1995) EMBO J. 14, 2816–2826.
[13] Starr, R., Willson, T.A., Viney, E.M., Murray, L.J., Rayner, J.R., Jenkins, B.J., Gonda, T.J., Alexander, W.S., Metcalf, D., Nicola, N.A. and Hilton, D.J. (1997) Nature 387, 917–921.
[14] Bjorbaek, C., Elmquist, J.K., Frantz, J.D., Shoelson, S.E. and Flier, J.S. (1998) Mol. Cell 1, 619–625.
[15] Al-Barazanji, K.A., Buckingham, R.E., Arch, J.R., Haynes, A., Mossakowska, D.E., McBay, D.L., Holmes, S.D., McHale, M.T., Wang, X.-M. and Gloger, I.S. (1997) Obes. Res. 5, 387–394.
[16] Emilsson, V., Liu, Y.-L., Cawthorne, M.A., Morton, N.N. and Davenport, M. (1997) Diabetes 46, 313–316.
[17] Liu, Y.-L., Emilsson, V. and Cawthorne, M.A. (1997) FEBS Lett. 411, 351–355.
[18] Islam, M.S., Morton, N.M., Hansson, A. and Emilsson, V. (1997) Biochem. Biophys. Res. Commun. 238, 851–855.
[19] Caldenhoven, E., van Dijk, T.B., Solari, R., Armstrong, J., Raaijmakers, J.A.M., Lammers, J.W.J., Koenderman, L. and de Groot, R.P. (1996) J. Biol. Chem. 271, 13221–13227.
[20] Maffei, M., Halaas, J., Ravussin, E., Pratley, R.E., Lee, G.H., Zhang, Y., Fei, H., Kim, S., Lallone, R., Ranganathan, S., Kern, P.A. and Friedman, J.M. (1995) Nature Med. 1, 1155–1161.
[21] Collins, S. and Surwit, R.S. (1996) J. Biol. Chem. 271, 9437–9440.
[22] Pelleymounter, M.A., Cullen, M.J., Baker, M.B., Hecht, R., Winters, D., Boone, T. and Collins, F. (1995) Science 269, 540–543.
[23] Vaisse, C., Halaas, J.L., Horvath, C.M., Darnell Jr., J.E., Stoffel, M. and Friedman, J.M. (1996) Nature Genet. 14, 95–97.
[24] Matsumoto, A., Masuhara, M., Mitsui, K., Yokouchi, M., Ohtsubo, M., Misawa, H., Miyajima, A. and Yoshimura, A. (1997) Blood 89, 3148–3154.