Isoproterenol is a positive regulator of the suppressor of cytokine signaling-3 gene expression in 3T3-L1 adipocytes

M Fasshauer, J Klein¹, U Lossner and R Paschke

University of Leipzig, Department of Internal Medicine III, 04103 Leipzig, Germany

¹Medical University of Lübeck, Department of Internal Medicine I, 23538 Lübeck, Germany

(Requests for offprints should be addressed to R Paschke; Email: pasr@medizin.uni-leipzig.de)

Abstract

SOCS (suppressor of cytokine signaling)-3 has recently been shown to be an insulin- and tumor necrosis factor (TNF)- α -induced negative regulator of insulin signaling. To further clarify a potential involvement of SOCS-3 in the development of insulin resistance, we measured differentiation-dependent SOCS-3 mRNA expression in 3T3-L1 adipocytes and studied its regulation by various hormones known to impair insulin signaling using quantitative real-time RT-PCR. There was a differentiation-dependent downregulation of SOCS-3 mRNA by 50% over the 9 day adipocyte differentiation course. Interestingly, besides insulin and TNF- α , chronic treatment of differentiated 3T3-L1 cells with 10 μ M isoproterenol for 16 h stimulated SOCS-3 gene expression by about 3.5-fold. Furthermore, isoproterenol stimulated SOCS-3 mRNA expression in a dose-dependent manner with significant activation detectable at concentrations as low as 10 nM isoproterenol. Moreover, a strong 27- and 47-fold

activation of SOCS-3 mRNA expression could be seen after 1 h of isoproterenol and GH treatment respectively. The stimulatory effect of isoproterenol could be almost completely reversed by pretreatment of 3T3-L1 cells with the β -adrenergic antagonist propranolol. Finally, isoproterenol's action could be mimicked by stimulation of G_s-proteins with cholera toxin and of adenylyl cyclase with forskolin and dibutyryl cAMP. Taken together, our results demonstrate a differentiation-dependent downregulation of SOCS-3 in adipocytes and suggest that SOCS-3 gene expression is stimulated by β -adrenergic agents via activation of a G_s-protein-adenylyl cyclase-dependent pathway. As SOCS-3 is a novel inhibitor of insulin signaling, the data support a possible role of this protein as a selectively regulated mediator of catecholamine-induced insulin resistance.

Journal of Endocrinology (2002) **175**, 727–733

Introduction

Insulin resistance, a diminished biological response of tissues to insulin, is a major risk factor for the development of type 2 diabetes, hypertension and coronary artery disease (Matthaei *et al.* 2000). At the cellular level, defects of various insulin signaling molecules are frequently observed in insulin resistance and are regarded as a major cause of this pathological condition. Thus, various studies have shown impaired activation of insulin receptor (IR), IR substrates (IRS), phosphatidylinositol (PI)-3 kinase and protein kinase B in insulin resistance and type 2 diabetes mellitus, leading to impaired activation of insulin-induced metabolic end points such as glucose uptake (Kahn & Flier 2000). Over the last couple of years, various circulating molecules such as free fatty acids (Bluher *et al.* 2001), insulin (Bluher *et al.* 2001), tumor necrosis factor (TNF)- α (Hotamisligil 1999), catecholamines (Bluher *et al.* 2000), angiotensin (AT) 2 (Folli *et al.* 1997), growth hormone (GH) (Takano *et al.* 2001) and glucocorticoids (Andrews

& Walker 1999) have been demonstrated to impair insulin signaling. However, molecules mediating these insulin resistance-inducing effects are only partly understood.

Suppressor of cytokine signaling (SOCS) proteins were originally identified as negative regulators of cytokine action. Several cytokines such as interleukin-2, -3 and -6, and interferon- γ induce SOCS expression in a tissue-specific manner (Endo *et al.* 1997, Naka *et al.* 1997, Starr *et al.* 1997). SOCS proteins modulate cytokine-activated Jak/Stat pathways by binding to either Jak or the cytoplasmic tail of cytokine receptors (Endo *et al.* 1997, Naka *et al.* 1997, Starr *et al.* 1997). Recently, it was demonstrated that insulin and TNF- α stimulate SOCS-3 gene expression in 3T3-L1 adipocytes and that SOCS-3 is increased in insulin resistance and obesity (Emanuelli *et al.* 2000, 2001). Most interestingly, it was shown that SOCS-3 inhibited insulin signaling (Emanuelli *et al.* 2000, 2001). Thus, the data accumulated so far suggest that SOCS-3 might be an important novel mediator of cellular

insulin resistance and a potential new target in the treatment of insulin resistance and obesity.

It appears possible that various hormones might decrease insulin sensitivity at least partly by upregulating SOCS-3. In the current study, we therefore examined the effect of differentiation and hormones such as isoproterenol, dexamethasone, AT2, GH and triiodothyronine (T3) on SOCS-3 gene expression in 3T3-L1 adipocytes *in vitro*. We demonstrate for the first time a differentiation-dependent inhibition and show that isoproterenol stimulates SOCS-3 mRNA expression in 3T3-L1 cells. Furthermore, we present evidence that the stimulatory effect of isoproterenol is mediated via β -adrenergic receptors, G_s -proteins and adenylyl cyclase.

Materials and Methods

Materials

Isoproterenol, TNF- α , insulin, dexamethasone, AT2, T3 and GH were purchased from Sigma Chemical Co. (St Louis, MO, USA). Cell culture reagents were obtained from Life Technologies, Inc. (Grand Island, NY, USA), oligonucleotides from MWG-Biotech (Ebersberg, Germany).

Culture and differentiation of 3T3-L1 cells

3T3-L1 adipocytes (American Type Culture Collection, Rockville, MD, USA) were cultured and differentiated as described (Fasshauer *et al.* 2001b,c). Briefly, confluent preadipocytes were cultured for 3 days in DMEM containing 25 mM glucose, 10% fetal bovine serum and antibiotics (culture medium) further supplemented with 1 μ M insulin, 0.5 mM isobutylmethylxanthine and 0.1 μ M dexamethasone, and for 3 days in culture medium with 1 μ M insulin. After additional 3–6 days in culture medium more than 90% of the cells had accumulated fat droplets.

Analysis of SOCS-3 gene expression

SOCS-3 gene expression was measured by quantitative real-time RT-PCR in a fluorescent temperature cycler (LightCycler; Roche Molecular Biochemicals, Mannheim, Germany) as previously described (Fasshauer *et al.* 2001d). Briefly, total RNA was isolated from 3T3-L1 adipocytes using TRIzol (Life Technologies, Inc., Grand Island, NY, USA) and 1 μ g RNA was reverse transcribed using standard reagents (Life Technologies). Ten percent of each RT reaction was amplified in a 20 μ l PCR containing 3 mM $MgCl_2$, 0.5 μ M each primer and 1 $\times$ LightCycler DNA Master SYBR Green I mix (Roche). Samples were incubated in the LightCycler for an initial denaturation at 94 °C for 30 s, followed by

40 PCR cycles. Each cycle consisted of 95 °C for 1 s, 61 °C for 7 s, and 72 °C for 11 s. The following primers were used: SOCS-3 (accession no. U88328) CCCTGC ACAGCCCTCCTTTCTCAC (sense) and GCCCCAC CCAGCCCCATACC (antisense); 36B4 (accession no. NM007475) AAGCGCGTCCTGGCATTGTCT (sense) and CCGCAGGGGAGCAGTGGT (antisense). SYBR Green I fluorescence emissions were monitored after each cycle and mRNA levels of SOCS-3 and 36B4 were quantified by using the second-derivative maximum method of the LightCycler Software (Roche). This method determines the crossing points of individual samples by an algorithm identifying the first turning point of the fluorescence curve. SOCS-3 synthesis was expressed relative to 36B4, which has been frequently used as an internal control due to its resistance to hormonal regulation (Lin *et al.* 2002). Linearity between total RNA used per reaction and amount of mRNA measured by the LightCycler software was obtained in the range between 5 and 200 ng total RNA for SOCS-3 and 36B4 respectively (data not shown).

To confirm amplification of specific transcripts, melting curve profiles (cooling the sample to 68 °C and heating slowly to 95 °C with continuous measurement of fluorescence) were produced at the end of each PCR. The specificity of the PCR was further verified by subjecting the amplification products to agarose gel electrophoresis.

Statistical analysis

Results are shown as means $\pm$ S.E. For analysis of differences between various treatments, unpaired Student's *t*-tests were used. $P < 0.01$ is considered highly significant.

Results

SOCS-3 mRNA expression is inhibited during differentiation

SOCS-3 mRNA expression during differentiation was analyzed. As compared with confluent 3T3-L1 preadipocytes on day 0, SOCS-3 mRNA synthesis was inhibited by about 50% between days 6 and 9 of differentiation ($P < 0.01$) (Fig. 1A). In contrast, SOCS-3 gene expression was not inhibited but rather augmented if NIH-3T3 cells, which do not differentiate, were exposed to the adipocyte differentiation protocol (Fig. 1B).

Isoproterenol and TNF- α are potent stimulators of SOCS-3 gene expression

As SOCS-3 has recently been suggested as a potent negative regulator of insulin signaling, we tested whether various hormones which have been shown to induce insulin resistance might influence SOCS-3 gene expression in 3T3-L1 adipocytes *in vitro*. Interestingly, treatment

Figure 1 Differentiation-dependent SOCS-3 gene expression. Confluent 3T3-L1 (day 0 (A)) and NIH-3T3 cells (day 0 (B)) were differentiated as described in Materials and Methods. At the days indicated total RNA was subjected to quantitative real-time RT-PCR to determine SOCS-3 mRNA levels relative to cells on day 0 (=100%). Results are the means $\pm$ S.E. of two (A) and three (B) independent experiments. ** $P < 0.01$ comparing confluent cells (day 0) with differentiated adipocytes (day 6, 9).

of 3T3-L1 cells with 10 μ M isoproterenol and 10 ng/ml TNF- α for 16 h increased SOCS-3 mRNA expression by 3.5- and 2.7-fold respectively, as compared with untreated controls ($P < 0.01$) (Fig. 2). Furthermore, insulin dramatically and transiently increased SOCS-3 gene expression almost 7-fold by 1 h of stimulation (data not shown). In contrast, AT2 (10 μ M), dexamethasone (100 nM), GH (500 ng/ml) and T3 (1 μ M) did not significantly influence expression of SOCS-3 (Fig. 2).

Isoproterenol stimulated SOCS-3 gene expression in a dose-dependent fashion. Thus, a significant 1.7-fold increase of SOCS-3 mRNA was detectable at isoproterenol concentrations as low as 10 nM ($P < 0.01$) (Fig. 3). A maximal 6-fold increase was found at 1 μ M of the β -adrenergic agonist ($P < 0.01$) (Fig. 3). Furthermore, TNF- α induced SOCS-3 dose-dependently with a maximal stimulation detectable at 100 ng/ml effector (data not shown).

Figure 2 SOCS-3 gene expression is stimulated by isoproterenol and TNF- α . Fully differentiated 3T3-L1 adipocytes were serum-starved for 6 h before isoproterenol (Iso, 10 μ M), TNF- α (TNF, 10 ng/ml), AT2 (10 μ M), dexamethasone (Dexa, 100 nM), GH (500 ng/ml) and T3 (1 μ M) were added for 16 h. Total RNA was subjected to quantitative real-time RT-PCR to determine SOCS-3 mRNA levels normalized to 36B4 relative to untreated control (Co) cells (=100%) as described in Materials and Methods. Results are the means $\pm$ S.E. of at least four independent experiments. ** $P < 0.01$ comparing hormone-treated with non-treated cells.

Isoproterenol and GH acutely stimulate SOCS-3 gene expression

To determine acute regulation of SOCS-3 mRNA synthesis, 3T3-L1 adipocytes were treated for different periods of time with 10 μ M isoproterenol or 500 ng/ml GH. As shown in Fig. 4, maximal 27- and 47-fold activation of SOCS-3 gene expression could be detected 1 h after addition of isoproterenol and GH respectively. Interestingly, SOCS-3 mRNA expression returned to

Figure 3 Dose-dependent stimulation of SOCS-3 gene expression by isoproterenol. 3T3-L1 cells were serum-starved for 6 h before various concentrations of isoproterenol were added for 16 h. Total RNA was extracted and subjected to quantitative real-time RT-PCR to determine SOCS-3 mRNA levels normalized to 36B4 expression as described in Materials and Methods. Data are expressed relative to untreated control cells (=100%). Results are the means $\pm$ S.E. of at least two independent experiments. ** $P < 0.01$ comparing effector-treated with non-treated cells.

A

B

Figure 4 SOCS-3 mRNA is acutely upregulated by isoproterenol and GH. 3T3-L1 cells were serum-starved overnight before isoproterenol (10 μ M (A)) and GH (500 ng/ml (B)) were added for the times indicated. Total RNA extraction and quantitative real-time RT-PCR determining SOCS-3 mRNA levels relative to 36B4 expression were performed as described in Materials and Methods. Data are expressed relative to untreated control cells (=100%) and are the means $\pm$ S.E. of three independent experiments. ** $P < 0.01$ comparing effector-treated with non-treated cells.

basal levels after 8 h GH treatment, whereas it remained upregulated in isoproterenol-treated cells (Fig. 4A and B).

The stimulatory effect of isoproterenol on SOCS-3 mRNA expression is mediated via β -adrenergic receptors, G_s -proteins and adenylyl cyclase

To verify that stimulation of SOCS-3 gene expression by isoproterenol is, in fact, mediated via β -adrenergic receptors, fully differentiated 3T3-L1 cells were pretreated with selective antagonists of α - (phentolamine, 100 μ M) and β - (propranolol, 100 μ M) adrenergic receptors for 1 h before isoproterenol (10 μ M) was added for 16 h. SOCS-3 mRNA expression significantly increased 3.8-fold after addition of isoproterenol as compared with untreated control cells ($P < 0.01$) (Fig. 5). This increase was com-

Figure 5 Stimulation of SOCS-3 gene expression by isoproterenol is mediated via β -adrenergic receptors. After 3T3-L1 cells were serum-starved for 5 h, adipocytes were cultured in the presence or absence of phentolamine (Phen, 100 μ M) or propranolol (Prop, 100 μ M) for 1 h before isoproterenol (10 μ M) was added for 16 h. Total RNA was extracted and subjected to quantitative real-time RT-PCR to determine SOCS-3 normalized to 36B4 expression as described in Materials and Methods. Data are expressed relative to non-treated control (Co) cells (=100%). Results are the means $\pm$ S.E. of four independent experiments. ** $P < 0.01$ comparing isoproterenol-treated with non-treated and propranolol-pretreated adipocytes.

pletely blocked after pretreatment of 3T3-L1 adipocytes with propranolol ($P < 0.01$), whereas phentolamine did not have any effect (Fig. 5). Taken together, these results suggest that isoproterenol stimulates SOCS-3 gene expression via β -adrenergic receptors.

Typically, stimulation of β -adrenergic receptors leads to activation of G_s -proteins which, in turn, activate adenylyl cyclase. To confirm that the stimulatory effect of isoproterenol on SOCS-3 gene expression is mediated via these signaling molecules, 3T3-L1 adipocytes were treated with cholera toxin (1000 ng/ml), an activator of G_s -proteins, forskolin (200 μ M), a direct stimulator of adenylyl cyclase, and dibutyryl cAMP (100 mM), a stable cAMP-analogue. As shown in Fig. 6, all three effectors significantly activated SOCS-3 gene expression between 2.4- and 3.9-fold ($P < 0.01$). These effects were dose-dependent with significant stimulation detectable at concentrations as low as 10 ng/ml cholera toxin, 2 μ M forskolin and 10 mM dibutyryl cAMP (data not shown).

Discussion

Insulin's metabolic effects are mediated through the IR. Various substrate molecules including IRS proteins bind to the activated IR and are phosphorylated by its kinase activity (Saltiel & Kahn 2001). Several studies using knock-out mice and cells derived from transgenic animals have demonstrated that activation of IRS proteins is essential for insulin action (Araki *et al.* 1994, Tamemoto

Figure 6 Stimulation of SOCS-3 gene expression by isoproterenol is mediated via G_s -proteins and adenylyl cyclase. After 6 h serum-starvation, 3T3-L1 adipocytes were cultured for 16 h with isoproterenol (Iso, 10 μ M), cholera toxin (Cholera, 1000 ng/ml), forskolin (For, 200 μ M), and dibutyryl-cAMP (cAMP, 100 mM). Total RNA was extracted and quantitative real-time RT-PCR was performed as described in Materials and Methods. SOCS-3 mRNA levels normalized to 36B4 are expressed relative to non-treated control (Co) cells (=100%). Results are the means $\pm$ S.E. of four independent experiments. ** $P < 0.01$ comparing non-treated with isoproterenol-, cholera toxin-, forskolin- and dibutyryl-cAMP-treated cells.

et al. 1994, Withers *et al.* 1998, Fasshauer *et al.* 2000, 2001a). Furthermore, it has been demonstrated that insulin resistance is accompanied by decreased activation of several insulin signaling molecules, although the exact mechanisms are not completely understood (Matthaei *et al.* 2000).

There is growing evidence that catecholamines impair insulin sensitivity and that increased activity of the sympathetic nervous system contributes to insulin resistance (Facchini *et al.* 1996, Reaven *et al.* 1996, Landsberg 1999, Hoiegggen *et al.* 2000, Maisson *et al.* 2000). We and others have previously demonstrated impaired insulin signaling and impairment of insulin action after pretreatment of adipocytes with β -adrenergic agents (Klein *et al.* 1999, 2000). Thus, insulin-induced activation of IR, IRS-1 and PI-3 kinase, as well as insulin-stimulated glucose uptake, were impaired after pretreatment of adipocytes with β 3-adrenergic agonists (Klein *et al.* 1999). Similarly, activation of IR and IRS-1 and insulin-induced glucose uptake were blunted after pretreatment of adipocytes with β -adrenergic agents (Kirsch *et al.* 1983, Arsenis & Livingston 1986). In a clinical context, our group recently demonstrated that patients with pheochromocytoma are insulin resistant due to increased serum levels of catecholamines and that insulin resistance could be reduced by surgical removal of the tumors in most cases (Bluher *et al.* 2000). However, the proteins mediating inhibition of insulin signaling after β -adrenergic activation are still unclear. In the present study, we demonstrate for the first time that isoproterenol increases SOCS-3 gene expression in 3T3-L1 adipocytes. Recently, Emanuelli *et al.* (2000,

2001) demonstrated convincingly that SOCS-3 is a novel negative regulator of insulin signaling. The authors suggested competitive binding of SOCS-3 to phosphotyrosine 960 of the IR, which impairs insulin-induced activation of IRS-1 and Stat5b thereby inducing insulin resistance. Based on these observations, β -adrenergic up-regulation of SOCS-3 may be an important new mechanism by which isoproterenol impairs insulin sensitivity. Moreover, upregulation of SOCS-3 in obesity and insulin resistance might not only be mediated by insulin and TNF- α (Emanuelli *et al.* 2000, 2001) but also via β -adrenergic activation. Interestingly, Madiehe *et al.* (2001) recently demonstrated downregulation of SOCS-3 after adrenalectomy, which might at least partly be mediated via decreased levels of catecholamines. In the present study, we show evidence that isoproterenol stimulates SOCS-3 expression via activation of G_s -proteins and adenylyl cyclase, in accord with the classical view of β -adrenergic receptors being G_s -protein-coupled (Collins & Surwit 2001). Gasperini *et al.* (2002) recently demonstrated convincingly that SOCS-3 gene expression was upregulated in leukocytes after cholera toxin, forskolin and dibutyryl-cAMP treatment. Interestingly, inhibition of protein kinase A (PKA) did not reverse dibutyryl-cAMP-induced SOCS-3 synthesis, indicating that increased cAMP levels might mediate their positive effect on SOCS-3 expression independently of PKA. Furthermore, we present evidence that GH acutely and transiently upregulates SOCS-3 mRNA expression in 3T3-L1 adipocytes, consistent with data obtained in 3T3-F442A cells (Adams *et al.* 1998). Thus, transient upregulation of SOCS-3 expression by GH might be one mechanism by which GH impairs insulin signaling (Takano *et al.* 2001).

Increased serum levels of AT2, glucocorticoids or thyroid hormones have also been shown to impair glucose tolerance profoundly. At the molecular level, AT2 causes decreased activity of insulin signaling proteins such as IRS molecules, which we and others have shown to be essential for insulin action (Folli *et al.* 1997, Fasshauer *et al.* 2000, 2001a). Dexamethasone induces insulin resistance at least partly via downregulation of IRS-1 (Turnbow *et al.* 1994). Thyroid hormone-induced insulin resistance is accompanied by decreased expression and translocation of the insulin-responsive glucose transporter-4 (Fickova *et al.* 1997). In our *in vitro* system, chronic incubation of 3T3-L1 adipocytes with pharmacological doses of dexamethasone, AT2 and T3 does not influence SOCS-3 gene expression. Therefore, our data do not support a major role for SOCS-3 in insulin resistance induced by these hormones; however, we cannot exclude the possibility that they might alter SOCS-3 mRNA expression after different incubation periods or in different cellular systems.

In summary, we demonstrate for the first time that isoproterenol significantly stimulates SOCS-3 gene expression in 3T3-L1 adipocytes. Furthermore, we show evidence that isoproterenol exerts its effects via

β -adrenergic receptors, G_s -proteins and adenylyl cyclase. Moreover, insulin, TNF- α and GH are also positive regulators of SOCS-3 synthesis. These data indicate that upregulation of SOCS-3 is a selectively regulated mechanism that might constitute an important element in the pathogenesis of insulin resistance and the insulin resistance syndrome.

Acknowledgement

This work was supported by a grant of the FORMEL1 program of the University of Leipzig to M F.

References

- Adams TE, Hansen JA, Starr R, Nicola NA, Hilton DJ & Billestrup N 1998 Growth hormone preferentially induces the rapid, transient expression of SOCS-3, a novel inhibitor of cytokine receptor signaling. *Journal of Biological Chemistry* **273** 1285–1287.
- Andrews RC & Walker BR 1999 Glucocorticoids and insulin resistance: old hormones, new targets. *Clinical Science* **96** 513–523.
- Araki E, Lipes MA, Patti ME, Bruning JC, Haag B, Johnson RS & Kahn CR 1994 Alternative pathway of insulin signalling in mice with targeted disruption of the IRS-1 gene. *Nature* **372** 186–190.
- Arsenis G & Livingston JN 1986 Isoproterenol reduces insulin stimulation of hexose uptake by rat adipocytes via a postinsulin binding alteration. *Endocrinology* **119** 50–57.
- Bluher M, Windgassen M & Paschke R 2000 Improvement of insulin sensitivity after adrenalectomy in patients with pheochromocytoma. *Diabetes Care* **23** 1591–1592.
- Bluher M, Kratzsch J & Paschke R 2001 Plasma levels of tumor necrosis factor- α , angiotensin II, growth hormone, and IGF-I are not elevated in insulin-resistant obese individuals with impaired glucose tolerance. *Diabetes Care* **24** 328–334.
- Collins S & Surwit RS 2001 The beta-adrenergic receptors and the control of adipose tissue metabolism and thermogenesis. *Recent Progress in Hormone Research* **56** 309–328.
- Emanuelli B, Peraldi P, Filloux C, Sawka-Verhelle D, Hilton D & Van Obberghen E 2000 SOCS-3 is an insulin-induced negative regulator of insulin signaling. *Journal of Biological Chemistry* **275** 15985–15991.
- Emanuelli B, Peraldi P, Filloux C, Chavey C, Freidinger K, Hilton DJ, Hotamisligil GS & Van Obberghen E 2001 SOCS-3 inhibits insulin signaling and is up-regulated in response to tumor necrosis factor- α in the adipose tissue of obese mice. *Journal of Biological Chemistry* **276** 47944–47949.
- Endo TA, Masuhara M, Yokouchi M, Suzuki R, Sakamoto H, Mitsui K, Matsumoto A, Tanimura S, Ohtsubo M, Misawa H *et al.* 1997 A new protein containing an SH2 domain that inhibits JAK kinases. *Nature* **387** 921–924.
- Facchini FS, Stoohs RA & Reaven GM 1996 Enhanced sympathetic nervous system activity. The linchpin between insulin resistance, hyperinsulinemia, and heart rate. *American Journal of Hypertension* **9** 1013–1017.
- Fasshauer M, Klein J, Ueki K, Kriauciunas KM, Benito M, White MF & Kahn CR 2000 Essential role of insulin receptor substrate-2 in insulin stimulation of Glut4 translocation and glucose uptake in brown adipocytes. *Journal of Biological Chemistry* **275** 25494–25501.
- Fasshauer M, Klein J, Kriauciunas KM, Ueki K, Benito M & Kahn CR 2001a Essential role of insulin receptor substrate 1 in differentiation of brown adipocytes. *Molecular and Cellular Biology* **21** 319–329.
- Fasshauer M, Klein J, Neumann S, Eszlinger M & Paschke R 2001b Adiponectin gene expression is inhibited by beta-adrenergic stimulation via protein kinase A in 3T3-L1 adipocytes. *FEBS Letters* **507** 142–146.
- Fasshauer M, Klein J, Neumann S, Eszlinger M & Paschke R 2001c Isoproterenol inhibits resistin gene expression through a G(S)-protein-coupled pathway in 3T3-L1 adipocytes. *FEBS Letters* **500** 60–63.
- Fasshauer M, Klein J, Neumann S, Eszlinger M & Paschke R 2001d Tumor necrosis factor alpha is a negative regulator of resistin gene expression and secretion in 3T3-L1 adipocytes. *Biochemical and Biophysical Research Communications* **288** 1027–1031.
- Fickova M, Zorad S & Macho L 1997 The effect of *in vivo* thyroxine treatment on insulin receptors, glucose transport and GLUT4 in rat adipocytes. *Hormone and Metabolic Research* **29** 16–19.
- Folli F, Kahn CR, Hansen H, Bouchie JL & Feener EP 1997 Angiotensin II inhibits insulin signaling in aortic smooth muscle cells at multiple levels. A potential role for serine phosphorylation in insulin/angiotensin II crosstalk. *Journal of Clinical Investigation* **100** 2158–2169.
- Gasperini S, Crepaldi L, Calzetti F, Gatto L, Berlato C, Bazzoni F, Yoshimura A & Cassatella MA 2002 Interleukin-10 and cAMP-elevating agents cooperate to induce suppressor of cytokine signaling-3 via a protein kinase A-independent signal. *European Cytokine Network* **13** 47–53.
- Hoieggan A, Fossum E, Nesbitt SD, Palmieri V & Kjeldsen SE 2000 Blood viscosity, plasma adrenaline and fasting insulin in hypertensive patients with left ventricular hypertrophy. ICARUS, a LIFE Substudy. Insulin CARotids US Scandinavica. *Blood Pressure* **9** 83–90.
- Hotamisligil GS 1999 The role of TNF α and TNF receptors in obesity and insulin resistance. *Journal of Internal Medicine* **245** 621–625.
- Kahn BB & Flier JS 2000 Obesity and insulin resistance. *Journal of Clinical Investigation* **106** 473–481.
- Kirsch DM, Baumgarten M, Deufel T, Rinninger F, Kemmler W & Haring HU 1983 Catecholamine-induced insulin resistance of glucose transport in isolated rat adipocytes. *Biochemical Journal* **216** 737–745.
- Klein J, Fasshauer M, Ito M, Lowell BB, Benito M & Kahn CR 1999 Beta(3)-adrenergic stimulation differentially inhibits insulin signaling and decreases insulin-induced glucose uptake in brown adipocytes. *Journal of Biological Chemistry* **274** 34795–34802.
- Klein J, Fasshauer M, Benito M & Kahn CR 2000 Insulin and the beta3-adrenoceptor differentially regulate uncoupling protein-1 expression. *Molecular Endocrinology* **14** 764–773.
- Landsberg L 1999 Role of the sympathetic adrenal system in the pathogenesis of the insulin resistance syndrome. *Annals of the New York Academy of Sciences* **892** 84–90.
- Lin J, Puigserver P, Donovan J, Tarr P & Spiegelman BM 2002 Peroxisome proliferator-activated receptor gamma coactivator 1 beta (PGC-1 beta), a novel PGC-1-related transcription coactivator associated with host cell factor. *Journal of Biological Chemistry* **277** 1645–1648.
- Madiehe AM, Lin L, White C, Braymer HD, Bray GA & York DA 2001 Constitutive activation of STAT-3 and downregulation of SOCS-3 expression induced by adrenalectomy. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* **281** R2048–R2058.
- Maison P, Byrne CD, Hales CN & Wareham NJ 2000 Hypertension and its treatment influence changes in fasting nonesterified fatty acid concentrations: a link between the sympathetic nervous and the metabolic syndrome? *Metabolism* **49** 81–87.
- Matthaei S, Stumvoll M, Kellner M & Haring HU 2000 Pathophysiology and pharmacological treatment of insulin resistance. *Endocrine Reviews* **21** 585–618.

- Naka T, Narazaki M, Hirata M, Matsumoto T, Minamoto S, Aono A, Nishimoto N, Kajita T, Taga T, Yoshizaki K *et al.* 1997 Structure and function of a new STAT-induced STAT inhibitor. *Nature* **387** 924–929.
- Reaven GM, Lithell H & Landsberg L 1996 Hypertension and associated metabolic abnormalities – the role of insulin resistance and the sympathoadrenal system. *New England Journal of Medicine* **334** 374–381.
- Saltiel AR & Kahn CR 2001 Insulin signalling and the regulation of glucose and lipid metabolism. *Nature* **414** 799–806.
- Starr R, Willson TA, Viney EM, Murray LJ, Rayner JR, Jenkins BJ, Gonda TJ, Alexander WS, Metcalf D, Nicola NA *et al.* 1997 A family of cytokine-inducible inhibitors of signalling. *Nature* **387** 917–921.
- Takano A, Haruta T, Iwata M, Usui I, Uno T, Kawahara J, Ueno E, Sasaoka T & Kobayashi M 2001 Growth hormone induces cellular insulin resistance by uncoupling phosphatidylinositol 3-kinase and its downstream signals in 3T3-L1 adipocytes. *Diabetes* **50** 1891–1900.
- Tamemoto H, Kadowaki T, Tobe K, Yagi T, Sakura H, Hayakawa T, Terauchi Y, Ueki K, Kaburagi Y & Satoh S 1994 Insulin resistance and growth retardation in mice lacking insulin receptor substrate-1. *Nature* **372** 182–186.
- Turnbow MA, Keller SR, Rice KM & Garner CW 1994 Dexamethasone down-regulation of insulin receptor substrate-1 in 3T3-L1 adipocytes. *Journal of Biological Chemistry* **269** 2516–2520.
- Withers DJ, Gutierrez JS, Towery H, Burks DJ, Ren JM, Previs S, Zhang Y, Bernal D, Pons S, Shulman GI *et al.* 1998 Disruption of IRS-2 causes type 2 diabetes in mice. *Nature* **391** 900–904.

Received 24 July 2002

Accepted 8 August 2002