Fatty acids inhibit leptin signalling in BRIN-BD11 insulinoma cells

C P Briscoe, S Hanif¹, J R S Arch and M Tadayyon

Department of Vascular Biology, SmithKline Beecham Pharmaceuticals, New Frontiers Science Park, Harlow, Essex, UK

¹Division of Biochemistry and Molecular Biology, University of Glasgow, Glasgow G12 8QQ, UK

(Requests for offprints should be addressed to M Tadayyon)

ABSTRACT

The effect of treatment with a 0.03% fatty acid (FA) cocktail on leptin-receptor-mediated STAT (signal transducers and activators of transcription) activation in the rat insulinoma cell line BRIN-BD11 was investigated. Leptin (10 nM) stimulated the tyrosine phosphorylation of STAT3 and STAT5b. Acute treatment with FAs prevented leptin-stimulated STAT3 tyrosine phosphorylation and significantly raised basal STAT5 phosphorylation. A chronic treatment (5 days) of BRIN-BD11 cells with FAs similarly attenuated leptin-stimulated STAT tyrosine phosphorylation. Chronic FA treatment also attenuated prolactin-stimulated STAT5b tyrosine phosphorylation but not

interleukin-6-stimulated STAT3 tyrosine phosphorylation, suggesting that the effect is receptor/ligand specific. TaqMan analysis of gene expression following chronic FA treatment showed neither a decrease in the amount of leptin receptor (Ob-R) mRNA, nor an increase in the negative regulators of STAT signalling, SOCS3 (suppressors of cytokine signalling) or cytokine inducible sequence (CIS). These data demonstrate that FAs modulate leptin and prolactin signalling in β -cells, implying that high levels of circulating FAs present in obese individuals affect the action of selective cytokines in β -cell function.

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INTRODUCTION

The adipocyte-derived hormone leptin inhibits food intake and raises energy expenditure through receptors present in the hypothalamic region of the brain (Zhang *et al.* 1994, Chen *et al.* 1996, Lee *et al.* 1996). The leptin receptor (Ob-R) belongs to the class 1 cytokine receptor superfamily (Baumann *et al.* 1996), which signal through JAKs and STATs (Heinrich *et al.* 1998). Ob-R is alternatively spliced (Tartaglia *et al.* 1995), the longest variant, Ob-Rb, being the JAK/STAT signalling and metabolically active form. Leptin activates STAT3 in mouse and rat hypothalamus to induce satiety (Vaisse *et al.* 1996, McCowen *et al.* 1998). Leptin-stimulated STAT activation has also been detected in peripheral tissues and cell lines endogenously expressing Ob-Rb, for example in islets from *ob/ob* mice lacking endogenous leptin (Seufert *et al.* 1999), in RINm5F insulinoma cells (Morton *et al.* 1999), in small-intestine epithelium and in CaCo2 cells (Morton *et al.* 1998). Although the shorter leptin

receptor isoforms may have signalling capacities, they are unable to activate the JAK/STAT pathway (Bjorbaek *et al.* 1997).

Leptin levels are elevated in obese individuals (Frederich *et al.* 1995, Considine *et al.* 1996), which leads to the proposal that these patients may have reduced leptin sensitivity. Elevated levels of the inhibitors of STAT signalling, a suppressor of cytokine signalling (SOCS) (Starr *et al.* 1997) and the cytokine inducible sequence (CIS) (Yoshimura *et al.* 1995), both of which are induced by STATs, have been implicated in obesity-related attenuation of leptin sensitivity. Overexpression of Ob-Rb and SOCS3 in Chinese hamster ovary (CHO) or COS-1 cells and of CIS in RINm5F cells was shown to inhibit leptin signalling (Bjorbaek *et al.* 1998, Emilsson *et al.* 1999). *In vitro* leptin stimulates SOCS3 mRNA and protein expression in CHO cells overexpressing Ob-Rb (Bjorbaek *et al.* 1999), and *in vivo* peripheral administration of leptin for 2 h raises the SOCS3 level in the hypothalamus of the *ob/ob* mouse (Bjorbaek *et al.* 1998).

Of all of the peripheral tissues, it is total pancreas that has the highest proportion of Ob-Rb relative to Ob-Ra (Ghilardi *et al.* 1996). Leptin suppresses insulin release from pancreatic islets of *ob/ob* mice (Emilsson *et al.* 1997). Inhibitory effects of leptin on insulin secretion from insulinoma cells have also been reported, e.g. in RINm5F and β TC6 cells (Kulkarni *et al.* 1997, Zhao *et al.* 1998). Leptin also stimulates cell proliferation in insulinoma cells (Islam *et al.* 1997, Tanabe *et al.* 1997) and raises fatty acid (FA) oxidation and mRNA for enzymes involved in FA oxidation, thus lowering triglyceride content (Zhou *et al.* 1997, Shimabukuro *et al.* 1997).

FAs have been found to affect β -cell function through a number of mechanisms (Unger 1995). Studies in the pre-diabetic obese Zucker diabetic fatty (ZDF) rat, which is leptin insensitive because of a mutation in Ob-Rb, have shown that the early build up of β -cell triglyceride, due to the high levels of free FAs and increased lipogenesis (Lee *et al.* 1997), may contribute to the subsequent onset of early diabetic symptoms and eventual β -cell failure in this animal (Lee *et al.* 1994, 1997, Zhou & Grill 1994). As leptin is essential for regulation of β -cell FA metabolism, obese individuals having high levels of circulating FAs may be prone to developing β -cell defects if leptin sensitivity is compromised. Although FAs have been reported to bind to leptin, the effects on downstream signalling pathways have not been determined (Campbell *et al.* 1998, Hidaka *et al.* 1999). We have therefore investigated whether FAs can influence leptin-receptor-mediated STAT activation in the insulinoma cell line BRIN-BD11 and whether changes in Ob-Rb, SOCS3 or CIS might be involved in this process.

MATERIALS AND METHODS

Cell culture

BRIN-BD11 cells were a gift from Professor P Flatt (Coleraine, N. Ireland). Cells were maintained in RPMI-1640 medium (Gibco BRL, Paisley, UK) supplemented with 10% (v/v) foetal bovine serum (FBS), 2 mM glutamine and 5 mM glucose at 37 °C in 5% CO₂. For chronic FA treatment studies, cells were seeded at 4×10^5 cells/25-cm² flask in medium containing 2% FA-free BSA (Sigma Chemical Co., Gillingham, Kent, UK) and 0.03% FA mixture comprising methyl esters of myristic acid (11.8%), palmitic acid (23.6%), palmitoleic acid (6.9%), stearic acid (13.1%), oleic acid (44.6%) (Sigma Chemical Co.) or vehicle (0.045% ethanol). After 24 h, cells were rendered quiescent in medium

containing 2% BSA, 0.5% FBS and 0.03% FAs or vehicle control. For acute FA studies, cells were pretreated for 5 days exactly as described above for control cells.

TaqMan analysis of leptin-receptor expression

Complementary DNA-specific 5' nuclease assays for Ob-Ra and Ob-Rb were designed using Primer Express software, (PE Applied Biosystems, Warrington, UK). Primers (Genosys, Cambridge, UK) and probes (PE Applied Biosystems) were as follows: Ob-Ra; forward primer 5'-TGTTTGTGGACGATGTTCCA-3', reverse primer 5'-GATACCTTCAAAGAGTGTCCGCTCTC-3', probe 5'-CAAGAATTGTTTCCTGGGCACAAGGACTTAATT-3', Ob-Rb; forward primer 5'-TGTTTGGGACGATGT TCCA-3', reverse primer 5'-AAAGATGCTCAAATGTTTCAGGC-3', probe 5'-CAAGAATTGTTTCCTGGGCACAAGGACTTAATT-3'. TaqMan assays were performed essentially as described previously (Overbergh *et al.* 1999). TaqMan utilises a gene-specific probe containing both fluorescent and quencher tags, which hybridise to the cDNA between the two PCR primers. As PCR proceeds, the 5' nuclease activity of the *Taq* polymerase cleaves the probe, releasing the tag such that the fluorescence is proportional to the amount of cDNA present. The relative quantity of a specific gene transcript present in a sample is determined by comparing the signal from a sample with that from a standard curve obtained from cDNA from rat-brain poly A⁺ RNA (Clontech, Basingstoke, Hants, UK).

Preparation of whole-cell lysates, and Western blotting

Cells were treated in serum-free medium containing 0.1% BSA and 10 nM murine leptin (Al-Barazanji *et al.* 1997), 1 nM human prolactin (Sigma), 1 nM murine interleukin-6 (IL-6) (R&D Systems, Abingdon, UK) or PBS (Gibco BRL) as a control. For acute FA treatments, stimulations were performed in the presence or absence of 0.03% FAs. After treatment, cells were washed with ice-cold PBS containing 1 mM Na₃VO₄. Cells were lysed in buffer containing 50 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EGTA, 2 mM EDTA, 1% IGEPAL detergent (Sigma Chemical Co.), 100 mM NaF, 10 mM Na₄P₂O₇, 1 mM Na₃VO₄ and a protease cocktail inhibitor tablet (Boehringer Mannheim, Germany). Cell lysates were centrifuged for 20 min at 14 000 *g* and the resulting supernatant was snap-frozen in liquid N₂ and stored at -80 °C. The protein concentration

was determined using the Bio-Rad protein assay kit (Bio-Rad Laboratories, Hemel Hempstead, Herts, UK).

Immunoprecipitations were performed overnight using 0.4–1 mg fresh lysate, 1 µg polyclonal anti-STAT3 (C-20) or polyclonal anti-STAT5b (C-17) antibody, with 40 µl of a 25% slurry of protein A/agarose (all from Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). Immunoprecipitations were washed with buffer containing 50 mM Tris (pH 7.5), 150 mM NaCl and 1 mM Na₃VO₄, before denaturation at 90 °C with 2 × Novex sample buffer (Invitrogen, Groningen, The Netherlands). Immunoprecipitates were subjected to SDS-PAGE on Novex 4–12% Bis-Tris polyacrylamide gels (Invitrogen) and transferred to PVDF membrane (Millipore, Watford, UK). Membranes were blocked and incubated with polyclonal anti-phosphoSTAT3 antibody (New England Biolabs, Hitchin, Herts, UK) or with monoclonal anti-phosphoSTAT5 antibodies (Upstate Biotechnology, Lake Placid, NJ, USA), then subjected to detection with anti-mouse or anti-rabbit immunoglobulin G secondary alkaline phosphatase-conjugated antibodies (Amersham Pharmacia Biotech, Amersham, Bucks, UK). Blots were exposed to an enhanced chemi-fluorescence (ECF) substrate (Amersham Pharmacia Biotech) and the STAT phosphorylation was quantified, using the fluorescent signal, on a STORM system (Amersham Pharmacia Biotech). In order to normalise STAT phosphorylation to the amount of STAT protein, blots were stripped and reprobed with anti-STAT3 or anti-STAT5b antibodies.

Statistical analysis

Results are expressed as means ± S.E.M. For clarity, one experiment that is representative of three is shown. Chemi-fluorescence data from Western blots from three independent experiments were pooled and analysed by 2-way ANOVA, using STATISTICA, $P < 0.05$ being considered statistically significant.

RESULTS AND DISCUSSION

BRIN-BD11 insulinoma cells express long and short leptin-receptor isoforms

RT-PCR was used to study leptin-receptor expression in the glucose-responsive BRIN-BD11 cells (fusions between rat β-cells and RINm5F cells) (McClenaghan *et al.* 1996). Both Ob-Ra and Ob-Rb were found to be expressed (Fig. 1A) and

co-migrated with Ob-Ra and Ob-Rb present in rat hypothalamus. Hybridisation to an internal Taq-Man probe confirmed the identities of the products (Fig. 1B).

Leptin stimulates tyrosine phosphorylation of STAT3 and STAT5

We set out to examine if FAs affect immediate leptin-receptor-mediated signalling; we used BRIN-BD11 cells as a model system, as they express Ob-Rb and proliferate in response to leptin (0.01–10 nM; data not shown), thus demonstrating functional responsiveness. Activation of STATs was determined by using the ECF system to quantify tyrosine phosphorylation of the proteins in immunoprecipitates from whole-cell lysates. Leptin (10 nM) elicited significant increases in tyrosine phosphorylation of both STAT3 (Fig. 2A; 2-way ANOVA, $F = 9.7$, $df = 10$, $P < 0.05$) and STAT5b (Fig. 2B; 2-way ANOVA, $F = 23.8$, $df = 12$, $P < 0.01$) after 30 min stimulation. Although the tyrosine phosphorylation of STAT proteins by leptin in BRIN-BD11 cells was small (135% of control for STAT3 and 178% of control for STAT5, Fig. 2), this response was reproducible and significant over several experiments. No changes in STAT3 or STAT5b tyrosine phosphorylation were observed after 15-min or 60-min treatments (data not shown), indicating the transient nature of leptin-stimulated STAT phosphorylation.

Acute FA treatment inhibits leptin-stimulated STAT tyrosine phosphorylation

Initial experiments in which FAs were added directly with leptin to the cells demonstrated that this acute treatment was sufficient to block leptin-stimulated increases in STAT tyrosine phosphorylation (Fig. 2). The mechanisms behind the effect of FAs on STAT3 phosphorylation versus STAT5 phosphorylation appear to be different; although there was no significant effect of FAs on basal STAT3 tyrosine phosphorylation, in the same experiment FAs significantly elevated basal STAT5 tyrosine phosphorylation 162% above control treatments (2-way ANOVA, $F = 37.7$, $df = 12$, $P < 0.01$) (Fig. 2B). Paradoxically, in the presence of FAs, 10 nM leptin produced a significant (62%) decrease in STAT5b tyrosine phosphorylation below basal levels (2-way ANOVA, $F = 12.62$, $df = 12$, $P < 0.01$). These experiments therefore suggest that the presence of FAs can alter leptin signalling and therefore the action of leptin in BRIN-BD11 cells.

FIGURE 1. Expression of Ob-Rb and Ob-Ra in BRIN-BD11 cells. RT-PCR was employed to detect mRNA for the short and long leptin-receptor isoforms, using specific primers in rat hypothalamus and BRIN-BD11 cells. (A) Amplified PCR products detected by ethidium bromide staining of a 6% polyacrylamide gel. (B) Representative TaqMan amplification plot with leptin-receptor-specific probe.

It is possible that acute FA treatment may affect negative regulators of STAT activity, but such an event must be rapid, occurring independently of transcription. The constitutively present cytosolic tyrosine phosphatase SHP-2 (SH2-containing phosphatase) is activated by leptin, becoming bound to Ob-Rb after JAK2-mediated tyrosine phosphorylation of the receptor (Li & Friedman 1999). Experiments performed in cells containing mutant Ob-Rb unable to bind SHP-2 (Tyr⁹⁸⁵) and endogenous SHP-2 have shown that leptin-stimulated binding of SHP-2 to Ob-Rb does

not modulate STAT3 tyrosine phosphorylation (Carpenter *et al.* 1998, Banks *et al.* 2000), nor was basal STAT3 phosphorylation affected. It seems unlikely, therefore, that acute FA treatment would alter either basal or leptin-stimulated STAT tyrosine phosphorylation through an effect on SHP-2 activity alone. Although the mechanism behind the leptin-mediated suppression of STAT5 tyrosine phosphorylation thus remains to be determined, it is possible that it may reflect opposite actions of leptin and FAs on currently undefined regulators of STAT5 phosphorylation.

FIGURE 2. Acute FA treatment inhibits leptin-stimulated STAT3 tyrosine phosphorylation. BRIN-BD11 cells were treated with 10 nM leptin or vehicle for 30 min in the presence or absence of 0.03% FAs and 2% BSA. STAT3 (A) or STAT5 (B) immunoprecipitates from whole-cell lysates were Western-blotted (WB) with (A) anti-phosphoSTAT3-specific antibody (anti-PSTAT3), stripped and then reprobed with anti-STAT3 antibody, or (B) anti-phosphoSTAT5-specific antibody (anti-PSTAT5), stripped and then reprobed with anti-STAT5 antibody. Phosphorylation was quantified by ECF and normalised to the levels of STAT protein present. Results are expressed as means $\pm$ S.E.M. ($n=3$) and are from one experiment representative of three. $\square$, Vehicle; $\blacksquare$, 0.03% FAs. ** $P<0.01$.

Inhibition of leptin-stimulated STAT tyrosine phosphorylation following chronic FA treatment

In order to study the more chronic effects of FA treatment on BRIN-BD11 cells, 5 day pretreatments with FAs were performed. This was followed by an acute 30 min treatment with 10 nM leptin (stimulations were performed in the absence of FAs). Chronic FA pretreatment of cells prevented the leptin-mediated increase in both STAT3 and STAT5b tyrosine phosphorylation (Fig. 3). Similarly, chronic FA treatment prevented leptin-stimulated DNA binding of STAT3 or STAT5, as determined by electromobility shift assays (EMSAs)

(data not shown). In contrast to acute treatment, chronic FA treatment did not significantly alter basal levels of STAT3 or STAT5 tyrosine phosphorylation.

The observed effects of the 5 day FA pretreatment on BRIN-BD11 cells did not appear to be due to losses in cell viability. The FA mixture used in our experiments contained a total of 1 mM FAs, comprising approximately 250 μ M palmitate and 440 μ M oleate. This FA mix appeared to have a proliferative effect over the 5 day period of treatment in BRIN-BD11 cells, more protein typically being recovered from cells treated with FAs than those treated with vehicle. In support of this finding, normal islets cultured

FIGURE 3. Effect of chronic FA treatment on leptin-stimulated STAT3 tyrosine phosphorylation. BRIN-BD11 cells were treated with 10 nM leptin or vehicle for 30 min after chronic FA or vehicle treatment of cells. STAT3 (A) or STAT5 (B) immunoprecipitates were Western-blotted (WB) with (A) anti-phosphoSTAT3-specific antibody (anti-PSTAT3), stripped and then reprobbed with anti-STAT3 antibody, or (B) anti-phosphoSTAT5-specific antibody (anti-PSTAT5), stripped and then reprobbed with anti-STAT5 antibody. Phosphorylation was quantified by ECF and normalised to the levels of STAT protein present. Results are expressed as means $\pm$ S.E.M. ($n=3$) and are from one representative experiment of three. □, Vehicle; ■, 0.03% FAs. Analysis, by 2-way ANOVA, of pooled data from three independent experiments showed that in cells pretreated for 5 days with vehicle, a 30 min treatment with 10 nM leptin elicited a significant increase in tyrosine phosphorylation of STAT3 ($F=9.3$, $df=12$, $*P<0.05$, $**P<0.01$) and STAT5 ($F=6.4$, $df=12$, $*P<0.05$).

for 7 days in 2 mM FAs oleate:palmitate (2:1) showed a 3.2-fold increase in bromodeoxyuridine incorporation (Milburn *et al.* 1995). Moreover, oleate was able to stimulate c-fos expression and cell proliferation of INS-1 cells (Roche *et al.* 1999).

Effect of chronic FA treatment on IL-6- and prolactin-stimulated STAT tyrosine phosphorylation

In order to determine whether the effects of chronic FA treatment were limited to leptin-receptor-

mediated events or whether other cytokine receptors, also signalling through the JAK-STAT pathway, were affected, the activation of STAT3 by IL-6 and that of STAT5 by prolactin were examined. Incubation of islets with IL-6 has been shown to inhibit glucose-stimulated insulin secretion from rat islets (Southern *et al.* 1990). IL-6 is also an activator of STAT3 in many different cell types (Zhong *et al.* 1994, Heinrich *et al.* 1998). As shown in Fig. 4A, IL-6 stimulated an increase in STAT3 tyrosine phosphorylation (2-way ANOVA from three independent experiments: $F=154.2$, $df=12$, $P<0.01$), which was not significantly altered

FIGURE 4. Chronic FA treatment inhibits prolactin- but not IL-6-stimulated increases in STAT tyrosine phosphorylation. BRIN-BD11 cells were treated with 1 nM IL-6 (A) or 1 nM prolactin (PL) (B) or vehicle for 30 min after chronic FA or vehicle treatment of cells. STAT3 (A) or STAT5 (B) immunoprecipitates Western-blotted (WB) with (A) anti-phosphoSTAT3-specific antibody (anti-PSTAT3), stripped and then reprobed with anti-STAT3 antibody or (B) anti-phosphoSTAT5-specific antibody (anti-PSTAT5), stripped and then reprobed with anti-STAT5 antibody. Phosphorylation was quantified by ECF and normalised to the levels of STAT protein present. Results are expressed as means $\pm$ S.E.M. ($n=3$) and are from one representative experiment (of three). □, Vehicle; ■, 0.03% FAs; ** $P<0.01$.

in cells treated chronically with FAs. Prolactin has been shown to stimulate STAT5 nuclear translocation and insulin secretion in a glucose-dependent manner in INS-1 cells (Stout *et al.* 1997). In our study using BRIN-BD11 cells, a 30 min treatment with 1 nM prolactin elicited a significant increase in tyrosine phosphorylation of STAT5b, after a 5 day pretreatment with either vehicle (2-way ANOVA, $F=102$, $df=11$, $P<0.01$) or FAs (2-way ANOVA, $F=18.7$ $df=11$, $P<0.01$). However, in contrast to the finding with IL-6, prolactin-stimulated STAT5b tyrosine phosphorylation was significantly attenuated in BRIN-BD11 cells after the 5 day FA treatment (3-way ANOVA, $F=14.5$, $df=22$,

$P<0.01$). Again, similar results were achieved, at the level of DNA binding, using EMSA (data not shown). This suggests that the effects of FAs are receptor or ligand specific and not due to different signalling strengths, as both IL-6 and prolactin produced approximately equivalent increases in STAT tyrosine phosphorylation.

Effect of FA pretreatment on expression of Ob-Rb, SOCS3 and CIS

Possible sites of action for the modulatory effects of chronic FA treatment include changes in expression

of the functional leptin receptor and/or of inhibitors of cytokine signalling. Studies with *ob/ob* mice and overexpression studies with CHO cells and RINm5F cells have suggested that levels of SOCS3 and CIS play an important role in inhibition of leptin-stimulated STAT signalling (Bjorbaek *et al.* 1998, Emilsson *et al.* 1999). Therefore, it is anticipated that FAs might prevent leptin-activation of STAT proteins, by elevating endogenous levels of SOCS3 or CIS or by raising levels of the receptor itself. In order to determine the relative quantities of Ob-Rb, SOCS3 and CIS mRNA present after chronic FA treatment, TaqMan analysis, a quantitative PCR technique, was performed. Neither an increase in SOCS3 or CIS nor a decrease in leptin-receptor expression appears to be a feature of the inhibitory effects of chronic FA treatment on leptin-receptor-mediated signalling in BRIN-BD11 cells. As there was no change in basal levels of STAT5 tyrosine phosphorylation after chronic FA treatment, different mechanisms may underlie inhibition of leptin-stimulated STAT5 phosphorylation in acute and chronic FA studies. Further support for the fact that neither SOCS3, CIS nor tyrosine phosphatases are involved in the FA effects comes from the findings that IL-6-elicited increases in STAT3 activation were unaffected by the chronic FA pretreatment. Furthermore, merely adding FAs together with leptin for 30 min also inhibits leptin signalling (Fig. 2), which, as previously discussed, argues against a role for induction of STAT-inducible negative regulators of STAT signalling in the FA effects.

Possible mechanisms of FA-mediated inhibition of leptin-stimulated STAT phosphorylation

Hidaka *et al.* (1999) demonstrated that unsaturated FAs were able to interact with leptin, whereas saturated FAs had no effect. Furthermore, oleic acid (20 μ M) was shown to produce a small decrease in the binding of leptin, immobilised on a sensor chip, to recombinant leptin receptor. Similarly, Campbell *et al.* (1998) showed that reversible binding of radiolabelled FAs to leptin altered the pI and electrophoretic mobility of the protein. The question of whether FAs affect binding of leptin to its receptor in cells or *in vivo* has not been addressed. Moreover, in neither study was the effect of FAs on receptor-mediated downstream signalling investigated. Since oleic acid was the predominant FA in the mix used in our experiments, the possibility that interactions between FAs and leptin were responsible for the reductions in leptin signalling should

be considered. Leptin receptor binding experiments were not attempted in our studies, because of the inherent difficulties in detecting binding in cell lines expressing endogenous levels of leptin receptors. The only successful reports of leptin receptor binding have been in cells overexpressing the receptor (Baumann *et al.* 1996). Furthermore, previously published reports of endogenous long-form leptin-receptor expression and signalling in cells have not demonstrated leptin/receptor binding (Islam *et al.* 1997, Morton *et al.* 1998, 1999).

Several factors strengthen the proposal that the effects of FA treatment on leptin-receptor-mediated STAT signalling are not merely due to interaction with leptin. First, stimulations in cells chronically treated with FAs were performed in FA-free media containing 0.1% BSA. It is therefore unlikely that sufficient FAs remained in the flasks, following the medium change, to interact with leptin and to alter leptin/receptor interaction. Secondly, if the binding of FAs to leptin were inhibiting receptor activation of STAT proteins, longer periods of leptin treatment or higher concentrations of leptin might diminish the FA effect and permit leptin-mediated STAT signalling. As the effects of chronic FA treatment were not reversed after the use of higher concentrations of leptin (100 nM) or after longer periods (60 min) of treatment (data not shown), it seems probable that other mechanisms are central to the inhibitory effect. To our knowledge, there are no published reports demonstrating binding of FAs to prolactin, or indeed to any other known cytokines, which suggests that the FA-mediated attenuation of prolactin- and leptin-stimulated STAT activation had occurred via a novel mechanism.

The mechanism behind inhibition of leptin-stimulated STAT activation after acute and chronic FA treatment may be different. Certainly, the 30 min treatment of cells with FAs in the presence of 0.1% BSA compared with the 5 day treatment with FAs with 2% BSA may be expected to lead to more pronounced effects on cell signalling, because of the higher levels of free FAs present in the acute experiment. One consideration, as previously discussed, was the elevation of basal STAT5 tyrosine phosphorylation after acute, but not after chronic, FA treatment. A final argument against a role for FA/leptin interaction was the observed suppression of STAT5 tyrosine phosphorylation, to below basal levels, in response to leptin in the presence of FAs. This observation suggests that, under these conditions, some form of receptor signalling may be occurring, although such a suppression of basal STAT3 tyrosine phosphorylation was not observed.

Conclusions

The mechanism behind the inhibition of cytokine-mediated STAT activation by FAs, therefore, probably involves an intracellular mediator upstream of STAT activation, common to both the leptin and prolactin receptor signalling pathway. In particular, acute FA treatment experiments point to a regulator of basal STAT5 tyrosine phosphorylation in the downregulation of leptin-stimulated STAT5 activation.

In summary, we have shown that a 0.03% FA cocktail inhibited leptin- and prolactin- but not IL-6-stimulated STAT activation. Although direct interaction between FAs and leptin could play a role in our observations, the data from chronic FA-treatment experiments suggest that additional mechanisms are also involved. FAs, by reducing sensitivity to leptin and prolactin, might be expected to have a negative impact on β -cell proliferation and insulin secretion.

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