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β3-adrenergic receptor induction of adipocyte inflammation requires lipolytic activation of stress kinases p38 and JNK

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Abstract

Activation of β-adrenergic receptors (AR) in adipocytes triggers acute changes in metabolism that can alter patterns of gene expression. This work examined the mechanisms by which activation of hormone sensitive lipase (HSL) induces expression of inflammatory cytokines in adipocytes in vivo and model adipocytes in vitro. β3-AR activation in mice triggered expression of inflammatory genes CCL2, IL-6, and PAI-1, as well as endoplasmic reticulum (ER) stress markers GRP78 and CHOP. Pharmacological inhibition of HSL blocked induction of inflammatory genes, but not ER stress markers. Promoting intracellular accumulation of free fatty acids (FFAs) in 3T3-L1 adipocytes increased expression of inflammatory cytokines, whereas inhibiting ceramide synthesis partly blocked PAI-1 expression, but not IL-6. Induction of inflammatory markers in vivo and in vitro was preceded by phosphorylation of p38 and JNK, and inhibition of HSL prevented activation of these kinases. Experiments with pharmacological inhibitors of specific MAP kinases demonstrated the importance of p38 MAPK as a mediator of lipolysis-induced inflammation in vivo and in vitro. Together, these results demonstrate that FFAs liberated by HSL activate p38 and JNK, and p38 mediates pro-inflammatory cytokines expression in adipose tissue.

Keywords

Inflammation; β3-adrenergic receptor; lipolysis; free fatty acids; ceramide; hormone sensitive lipase; p38; JNK

1. Introduction

β3-adrenergic receptors (AR) are selectively expressed in mature adipocytes, and chronic activation of these receptors upregulates fatty acid catabolism and improves insulin sensitivity in rodent models of diabetes [1]. Acute activation of β3-AR, however, triggers an inflammatory response that down regulates expression of key adipocyte regulatory genes, such as PPARγ and Cebpa, and delays adaptive upregulation of oxidative genes [2]. How β3-AR activation produces inflammation is not fully understood, however, our previous work strongly indicates that the release of free fatty acids (FFAs) through the action of hormone sensitive lipase (HSL) plays a critical role [3].

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Adipose tissue inflammation is thought to play a central role in diet-induced insulin resistance; however, the proximal events that lead to this inflammation are poorly understood owing in part to the significant delay between introduction of the obesogenic diet and onset of tissue inflammation. In contrast, a single injection of the β 3-AR agonist CL 316,243 (CL) induces an acute inflammatory state in adipose tissue marked by the expression of inflammatory cytokines, followed by infiltration of leukocytes [2,4]. β 3-AR expression in adipose tissue is limited to mature fat cells, and this specificity, along with the speed of induction of inflammation, suggested a tractable model for investigating mechanisms of adipose tissue inflammation and exploring the interaction between fat cell metabolism and gene expression. Our results demonstrate that acute β 3-AR activation induces inflammation and endoplasmic reticulum (ER) stress in adipose tissue and in cultured 3T3-L1 adipocytes. Inflammation, but not ER stress, results from β -adrenergic activation of HSL, accumulation of intracellular FFAs, and subsequent activation of p38 MAP kinase.

2. Methods

2.1 Animal Studies

All animal protocols were approved by Institutional Animal Care and Use Committee (IACUC) and the Division of Laboratory Animal Resources (DLAR) at Wayne State University. Time course analysis of gene expression was performed on 8 week old male 129S1/SvImJ (129S) mice, with six mice per group. Mice were killed at the indicated time points and epididymal white adipose tissue (EWAT) was removed and placed in RNAlater (Ambion) and held at -80°C until processed. To examine the effects of pharmacological inhibition of HSL, 8 week old male C57BL/6 mice ($n=7-8$) were pretreated with 30 mg/kg of the selective HSL inhibitor BAY 59-9435, 4-isopropyl-3-methyl-2-[1-(3-(S)-methylpiperidin-1-yl)-methanoyl]-2H-isoxalo-5-one (BAY) [5], suspended in 0.5% methylcellulose or methylcellulose alone via oral gavage. After one hour, mice were injected intraperitoneally with 10 nmol of CL or H_2O , and killed 3 hr later and EWAT pads were removed and stored as above. For in vivo studies using the selective p38 inhibitor SB 203580 (SB; LC Labs), 8 week old male 129S mice ($n=5$) were pretreated twice with vehicle or SB (0.35 mg/100 μl / mouse as a 35% DMSO solution) 18 hr and 1 hr prior to a 10 nmol IP injection of CL or H_2O , and killed 3 hr later. For mRNA expression analysis, EWAT RNA was extracted in Trizol (Invitrogen), and then purified with an RNeasy Mini Kit (Qiagen). The expression pattern of various genes was determined by quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) analysis, as previously described [2]. Briefly, RNA (0.5 – 1.0 μg) was reverse transcribed into cDNA by using Superscript III (Invitrogen) and oligo dT primers. Twenty to fifty ng of cDNA was subjected to qPCR analysis using SYBR Green (Abgene) as the fluorophore, and expression levels were normalized to that of peptidylprolylisomerase A (PPIA) mRNA. IL-6 cDNA was amplified using forward and reverse primers 5'-AGTGGCTAAGGACCAAGACC-3'; and 5'-TCTGACCACAGTGAGGAATG-3', PAI-1 cDNA were quantified with 5'-CCTCTTCATGGGCCAAGT-3' and 5'-GGTAAGGAGGAGTTGCCTTC-3, MPO with 5'-ACCGTGTCTGAAGAACAACAT-3' and 5'-AATAGCACAGGAAGGCCAAT-3, CD3 with 5'-TCATTGCTGGACAGGATGGA-3' and 5'-GGGCTGGTAGAGCTGGTCATT-3, GRP78 cDNA were quantified with 5'-TGTTTCAGCCAATTATCAGCA-3' and 5'-TCAATTTTCTCCCAACGAAA-3, and CHOP cDNA were quantified with 5'-AAGGAGAAGGAGCAGGAGAA-3' and 5'-AGACAGACAGGAGGTGATGC-3. All other qPCR primers were previously described [4].

2.2 Cell culture and in vitro Assays

3T3-L1 cells were differentiated as previously described [3], and experiments were performed on cultures maintained as differentiated adipocytes for 13–14 days. Adipocytes were cultured overnight in DMEM (Invitrogen), and media was replaced with serum-free, phenol red-free, DMEM (Mediatech) containing 0.1 % fatty acid free bovine serum albumin (BSA) (Roche Diagnostics) unless indicated otherwise. In experiments with the selective HSL inhibitor BAY, cells were pretreated with 10 μ M BAY or DMSO control for 1 hr. For experiments with selective chemical inhibitors of serine palmitoyltransferase (SPT) (Myriocin, 10 μ M), p38 (SB, 10 μ M), JNK (SP600125, 10 μ M; LC Labs) and MEK1/2 (PD098059 25 μ M; Chemicon), cells were pretreated for 1 hr prior to isoproterenol treatment. For gene expression experiments, cells were then stimulated with 10 μ M isoproterenol or H₂O (control), or with TNF α (10 ng/ml) for 3 hr. RNA extraction was performed using Trizol reagent (Invitrogen) or a Nucleospin RNA II Kit (Macherey Nagel). cDNA was synthesized and quantified by qPCR as described above. For western blot analysis, cells were stimulated for indicated times or for 1 hr in experiments with BAY.

2.3 Protein isolation and Western blot analysis

Proteins from 3T3-L1 adipocytes were lysed in buffer containing 20 mM Tris (pH7.5), 150 mM NaCl, 1mM EDTA, 1mM EGTA, 1% Triton X-100 and protease (Roche) and phosphatase (Pierce) inhibitors. Lysates were solubilized for 15 min at 4°C and centrifuged at 16,000 $\times$ g for 10 min to clear lysate. The extracts were recovered and proteins were quantified by using the bicinchoninic acid method (Pierce). Adipose tissue was homogenized in RIPA lysis buffer (20mM Tris, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 1% SDS, 1mM EDTA) with inhibitors and treated as above. 50 μ g of total protein was loaded on 10% mini-gels (Pierce) and SDS-PAGE was performed under standard conditions. Resolved proteins were transferred to PVDF and membranes were blocked for 1 hr at room temperature in 5% powdered skim milk. Antibodies for p38 (#9212), phospho-p38 (#9215), JNK (#9252) and phospho-JNK (#9251) were from Cell Signaling Technologies and were probed at 4°C overnight in 5% BSA. Blots were then washed, incubated with a secondary donkey anti-rabbit HRP (Jackson immunological) diluted 1:5000, and visualized with SuperSignal West Dura substrate (Pierce). Digital images were captured to ensure that pixels were not over-saturated and quantified using a BioRad Quantity One imaging system.

2.4 Statistical analysis

Unless stated otherwise, results are expressed as means $\pm$ SEM from a minimum of three independent experiments. Statistical analyses were performed with GraphPad Prism 5 using one way or two-way ANOVA with Bonferroni post t-tests to determine significance within groups. Planned post-hoc comparisons for one-way ANOVA were performed using Bonferroni post t-test (equal variances) or Dunn's multiple comparison test (unequal variances), as indicated in the legends.

3. Results and Discussion

3.1 β 3-AR induction of adipose tissue inflammation requires HSL activity, but induction of ER stress markers does not

We first examined temporal pattern of gene expression following a single injection of the β 3-AR agonist CL on inflammatory cytokine expression in 129S mice (Fig. 1). The expression of pro-inflammatory cytokines CCL2, IL-6 and PAI-1 was significant at one hour and maximal at three hours. The maximal induction of inflammatory cytokines following a single injection of CL occurred without significant changes in levels of macrophage (F4/80),

neutrophil (MPO) or T-lymphocyte (CD3) markers (Fig. 1), indicating that the upregulation of CCL2, IL-6 and PAI-1 at three hours is a primary response of adipose tissue, and not a consequence of immune cell recruitment. Levels of MPO reached significant at six hours compared to control, suggesting that immune cells initially recruited to adipose tissue after a single injection of CL [2,4] might be polymorphonuclear neutrophils (PMNs) as has been shown to occur during diet induced obesity [6]. Regardless, this does not account for the effects seen at three hours; therefore we chose the three hour time point for further experiments.

β 3-adrenergic receptors are fat cell-specific, and occupancy of these receptors activates several signaling pathways and metabolic processes [1,2,7]. We previously proposed that induction of inflammation by β -AR activation was provoked by excessive production of FFAs, triggered by the PKA-dependent activation of HSL as opposed to a direct transcriptional event [3]. To further investigate the role of HSL, we examined the effects of BAY 59-9435 (BAY) on acute inflammatory gene expression and ER stress responses to β 3-AR activation. C57BL/6 mice were used in these experiments as this strain is widely used to investigate adipose tissue inflammation[8], and to demonstrate that the phenomenon is independent of strain. BAY is a highly selective pharmacological inhibitor of HSL [5] that does not inhibit other lipases [9] or the newly discovered adipose triglyceride lipase (ATGL) [3]. Both inflammation and ER stress have been implicated in causing insulin resistance and are thought to be mechanistically related [10,11]. Injection of CL induced the expression of inflammatory markers CCL2, IL-6 and PAI-1, as well as the ER stress markers GRP78 and CHOP in C57BL/6 mice (Fig. 2). Inhibition of HSL with BAY had no effect on the basal expression of markers of inflammation and ER stress (Fig. 2). However, inhibition of HSL prevented induction of inflammation by β 3 receptor activation, but not that of the ER stress response (Fig. 2). Interestingly, HSL inhibition tended to further upregulate GRP78 expression, but not that of CHOP after CL treatment. These data indicate that β 3-adrenergic induction of inflammation and ER stress involve distinct pathways and that inflammation requires mobilization of fatty acids by HSL, but ER stress does not.

3.2 β 3-AR mediated activation of p38 and JNK requires HSL in vivo

We next turned our attention to mechanisms that might mediate the action of HSL and focused specifically on p38 and JNK, which are known to be activated by FFAs [12,13]. Injection of CL in 129S mice triggered the rapid and transient phosphorylation of p38 MAPK (Fig. 3A, 3C), and the sustained phosphorylation of JNK (Fig. 3B, 3D). Kinase activation occurred as early as 15 min post injection (earliest time point analyzed), prior to induction of inflammatory gene expression, and is consistent with involvement as mediators of HSL-dependent adipose tissue inflammation. To explore this possibility, we examined whether inhibition of HSL blocked CL-induced activation of both p38 and JNK in C57BL/6 mice (Fig. 3E–G). CL treatment elevated phospho levels of p38 and JNK and inhibition of HSL prevented activation of these kinases by CL treatment (Fig. 3E–G). β 3-ARs have been reported to activate p38 in brown fat [14], and β -AR mediated activation of p38 has been previously shown in isolated rat adipocytes, with little or no effect on JNK, but the mechanism was unknown [15]. The activation of p38 and JNK in vivo in white fat of mice has not been previously reported.

3.3 HSL mediates the expression of inflammatory cytokines and activation of p38 and JNK in vitro

The in vivo experiments indicate that selective activation of adipocyte lipolysis triggers local inflammation, and that this process likely involves activation of p38 and/or JNK. Adipose tissue contains multiple cell types that might contribute to inflammatory gene expression. To explore β -adrenergic regulation of adipocyte inflammation, we turned to

3T3-L1 adipocytes as an in vitro model system to replicate the above in vivo results and to dissect signaling events downstream of HSL and FFAs. As shown in Fig. 4, treatment of 3T3-L1 adipocytes with the general β -AR agonist isoproterenol induced expression of inflammatory cytokines CCL2 and PAI-1, and this effect was blocked by pretreatment with BAY. Similar results were observed with CL on 3T3-L1 adipocytes (not shown), indicating that induction of inflammation is not specific to the β 3-AR, but a general phenomenon of β -adrenergic receptor activation. In contrast, BAY pretreatment did not block induction of ER stress markers (Fig. 4).

Interestingly, inhibition of HSL tended to enhance isoproterenol induction of GRP78 and CHOP. It is currently not known how blocking HSL might lead to increased ER stress marker expression, but might occur through increased DAG levels which are known to be elevated by HSL deficiency [16]. It is important to note that BAY is highly selective for HSL [3,9] and does not block induction of CCL2 and PAI1 by TNF α which also activates p38 and JNK [17] (supplemental figure 1A). BAY did not affect expression of IL-6 (Supplemental Fig. 1B), since the induction of IL-6 by β 3-AR activation is independent of PKA activation and may be activated by both p38 and PKC in this cell line [18].

We next tested if isoproterenol could activate p38 and JNK in 3T3-L1 adipocytes and whether this was mediated by HSL. β -AR agonist treatment rapidly increased phosphorylation of p38 which persisted at least two hours (Fig. 5A). Phosphorylated JNK was not detected until 30 min after isoproterenol treatment and was maximal after two hours (Fig. 5A). Selective inhibition of HSL with BAY blocked the activation of both p38 and JNK as detected by phospho-specific antibodies (Fig. 5B). Importantly, BAY did not affect the phosphorylation status of p38 and JNK (Fig. 5B) in the absence of stimulation (Fig. 5C, D). p38 has been previously shown to be activated by cAMP analogues in isolated rat adipocytes, suggesting the involvement of pathways downstream of PKA [15]. Together, these results indicate β -adrenergic stimulation of lipolysis activates p38 and JNK and triggers inflammation in a fat cell autonomous manner.

3.4 Inflammation during β -AR activation is enhanced by limiting FFA efflux and partially suppressed by blocking ceramide synthesis

Previous results established that inflammation mediated by activation of β 3-AR is independent of the extracellular receptor Toll-like receptor 4 (TLR4), suggesting that inflammation might occur by intracellular accumulation of FFAs [3]. To address this question the FFA acceptor bovine serum albumin (BSA) was systematically reduced in the media of 3T3-L1 adipocytes in order to limit fatty acid efflux and promote accumulation of intracellular fatty acids [19]. Reducing the concentration of BSA systematically reduced extracellular FFA concentration while increasing intracellular FFA levels (supplemental Fig. 2A and 2B). Lowering BSA dramatically enhanced expression of CCL2 and PAI-1 and this effect was eliminated by HSL inhibition (Fig. 6A).

The above results strongly indicate that intracellular FFA or FFA-derived metabolites trigger adipocyte inflammation. Ceramides are potential mediators that are generated from FFAs (palmitic acid) and have been implicated in adipose tissue inflammation [20,21]. To test potential involvement of ceramides, we examined the effects of myriocin, a selective inhibitor of serine palmitoyltransferase (SPT) [22]. Treatment of 3T3-L1 adipocytes with myriocin significantly reduced the expression of PAI-1 after β -AR activation, but did not affect basal levels (Fig. 6B) (CCL2 was not significantly upregulated in this experiment so the effects of myriocin are not reported). Myriocin did not reduce IL-6 expression as expected, since upregulation of this cytokine is independent of HSL in these cells (Fig. 6B). Importantly, treatment with myriocin did not affect lipolysis (Supplemental Fig. 3A). These results indicate that intracellular accumulation of FFAs and subsequent generation of

ceramide contributes to adipocyte inflammation. Whether other FFA-derived metabolites, such as fatty acyl-CoAs, also contribute to inflammatory signaling is an important topic of future research.

3.5 p38 mediates β 3-AR expression of pro-inflammatory cytokines in vitro and in vivo

In vivo and in vitro experiments established that HSL was important in activating JNK and p38. We assessed the role of MAP kinases in the expression of inflammatory cytokines during β -AR activation by using selective pharmacological inhibitors of p38, JNK, and ERK1/2 in cultured 3T3-L1 adipocytes. The selective p38 inhibitor SB203580 (SB) blocked the expression of CCL2 and partly inhibited that of PAI-1 (Fig. 7A). The JNK inhibitor SP600125 (SP) partly blocked the expression of PAI-1 and did not block expression of CCL2 (Fig. 7A). Combined treatment of both p38 and JNK inhibitors resulted in an additive reduction in the expression of PAI-1, but did not decrease the expression of CCL2 beyond that produced by SB above. SB or SP did not affect lipolysis (Supplemental Fig. 3B). We also evaluated the role of ERK1/2 which is known to be activated by the β -adrenergic receptor [23]. The MEK1/2 inhibitor PD098059 (PD) did not significantly reduce the expression of any of the inflammatory cytokines studies (Fig. 7B). How signals derived from intracellular fatty acids are detected and transduced to p38 and JNK is not known, but could involve ceramide activated protein kinase (CAP) for p38 [24] or mixed-lineage protein kinase 3 (MLK3) for JNK [25].

Having established p38 as the major regulator of inflammatory genes during β -AR activation in vitro, we tested if p38 regulated the expression of cytokines in vivo. Pretreatment with SB slightly elevated levels of CCL2, IL-6 and PAI-1. Nonetheless, SB completely prevented induction of CCL2 and IL6 by β 3-AR activation. PAI-1, which was inconsistently induced by CL, was not significantly inhibited by SB (Fig. 7C). These results agree with explants studies of human adipose tissue in which pharmacological inhibition of p38 reduced expression of inflammatory cytokines [26]. Furthermore, the in vitro and in vivo results suggest that p38 is a molecular link between lipolysis and the genetic effects of β 3-AR.

It is interesting to note that genetic deletion of HSL in mice results in a chronic inflammatory state in white adipose tissue [3,27,28]. Inflammation in HSL knockout mice occurs through the recruitment of macrophage to dying hypertrophied adipocytes [27]. In contrast, our results demonstrate that acute inhibition of HSL prevented β -AR mediated inflammation in white adipose tissue and suggests that tightly controlling the pool of FFAs by preventing intracellular accumulation might be beneficial.

Mounting evidence indicates that disruption of the balance between fatty acid storage and mobilization in adipose tissue contributes to local and systemic inflammation [29], and is likely to play a role in obesity-induced inflammation [30]. The present results support the concept that modifying lipid flux in adipocytes towards intracellular FFAs promotes inflammation [20,31]. Interestingly, nicotinic acid, an inhibitor of adipocyte lipolysis and an agent used to improve lipid profile in the clinic, also has anti-inflammatory properties in fat cells [32]. Lipolysis per se is not detrimental provided fatty acid oxidation is increased to counter the increased FFA flux [33–35]. In this regard we note that chronic activation of β 3-AR dramatically expands mitochondrial fatty acid oxidation in white fat, and this expansion appears to be critical for limiting inflammation and improving insulin action [1,4]. Conversely, metabolic and inflammatory profiles can be improved by diverting FFAs into triglyceride droplets for storage [36]. Additionally, the anti-diabetic thiazolidinediones (TZDs) are known to promote adipogenesis and lipogenesis and also have anti-inflammatory properties in adipose tissue [37–39]. In summary this study reveals that lipolysis via activation of p38 induces inflammation in adipose tissue. Furthermore, the results suggest

that adipose tissue inflammation might be targeted by selective inhibitors of HSL or p38 MAPK. Alternatively, preventing accumulation of intracellular FFAs within adipocytes by either promoting storage into triglyceride or by promoting FFA oxidation in adipose tissue might be an additional means of preventing inflammation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

AR	adrenergic receptor
FFAs	free fatty acids
HSL	hormone sensitive lipase
ER	endoplasmic reticulum
CL	CL 316, 243
BAY	BAY 59-9435
JNK	c-Jun N-terminal Kinase
SB	SB 203580
SP	SP600125
BSA	bovine serum albumin
PPIA	peptidylprolyl isomerase A
IL-6	interleukin-6
CCL2	chemokine (C-C motif) ligand
PAI-1	plasminogen activator inhibitor-1
PKA	protein kinase A
PKC	protein kinase C
SPT	serine palmitoyl transferase

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Fig. 1.

Time course expression of inflammatory and immune cell markers. Mice were treated with a single injection of CL for indicated times and EWAT RNA was analyzed for mRNA expression by normalizing to % PPIA. Statistical significance by Dunn's test for CL treatment compared to time zero is indicated (* P<0.05, ** P<0.01).

Fig. 2.

Role of HSL during acute β 3-AR induced gene expression in adipose tissue. C57BL/6 mice were pretreated with BAY or methylcellulose (MC) for 1 hr, followed by CL treatment (10 nmol) or H₂O for 3 hr and EWAT was analyzed for mRNA expression. Statistical significance for CL treatment between groups is indicated (*** P<0.001; ** P<0.01; * P<0.05).

Fig. 3. BAY blocks the activation of p38 and JNK in EWAT during β 3-AR activation. Time course of p38 (A) and JNK (B) activation during β 3-AR agonist treatment in 129S1/SvImJ mice. Mice were treated for indicated times with CL and EWAT was analyzed by immunoblotting for phospho-specific antibodies against p38 (p38-P) and JNK (JNK-P), and total p38 (p38-T) and JNK (JNK-T). Densitometry was performed on three mice per time-point and expressed as the ratio of phospho/total for p38 (C) and JNK (D). (E), Mice were pretreated with MC or BAY for 1 hr followed by 10 nmol CL for 30 min and EWAT was analyzed by immunoblotting for phospho-specific antibodies and total antibodies. Densitometry was performed and phospho-levels were normalized to total p38 (F) and JNK (G) and statistical significance of CL treatment in the groups is noted (** $P < 0.01$; * $P < 0.05$).

Fig. 4.

Role for HSL during β -AR-induced gene expression in 3T3-L1 adipocytes. 3T3-L1 cells were pretreated with BAY or DMSO for 1 hr, followed by the β -AR agonist isoproterenol (Iso, 10 μ M) or H₂O for 3 hr. RNA was extracted from cells, cDNA synthesized and qPCR performed on indicated genes by normalizing to % PPIA, and then expressed as % Control (Ctl). Significance for Iso treatment within the groups is shown (*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$).

Fig. 5. BAY blocks the activation of p38 and JNK during β -AR stimulation in 3T3-L1 adipocytes. (A), Time course of p38 and JNK activation during β -AR stimulation. Cells were treated for indicated times with isoproterenol and lysates were immunoblotted for phospho-specific antibodies against p38 (p38-P) and JNK (JNK-P). Immunoblots were stripped and reprobed for total p38 (p38-T) and JNK (JNK-T). Representative blot of 2–3 experiments. (B), 3T3-L1 cells were pretreated with DMSO or 10 μ M BAY for 1 hr followed by 10 μ M isoproterenol (Iso) treatment for 1 hr and proteins analyzed as in A. Representative blot of three separate experiments. (C, D), quantification of immunoblots from three independent experiments from (B). Densitometry was performed and phospho-levels were normalized to total p38 (C) and JNK (D) to determine statistical significance for Iso treatment within the groups (** $P < 0.01$; * $P < 0.05$).

Fig. 6. β -AR mediated inflammation is promoted by intracellular buildup of FFAs and ceramide. (A), 3T3-L1 adipocytes were incubated in indicated concentrations of BSA, and pretreated with BAY or vehicle for 1 hr, followed by Iso (10 μ M) for 3 hr, and mRNA was analyzed for expression of indicated genes by normalizing to % Control (Ctl). Statistical significance is shown for comparisons of Ctl with different BSA concentrations (*** $P < 0.001$; ** $P < 0.01$). (B), 3T3-L1 adipocytes were pretreated with the SPT inhibitor myriocin (10 μ M) for 1 hr, followed by Iso treatment for 3 h, and mRNA was analyzed for expression of indicated genes. Results from B are from two independent experiments with technical duplicates and significance between comparisons is shown (* $P < 0.05$; ns, non significant).

Fig. 7. Role of p38 and JNK in CCL2, IL-6 and PAI-1 mRNA expression during β -AR activation in 3T3-L1 adipocytes. (A), 3T3-L1 cells were pretreated for 1 hr with the indicated inhibitors against p38 (SB, SB203580) and JNK (SP, SP600125) alone or in combination, followed by Iso treatment for 3 hr and mRNA expression was quantified for indicated genes. Statistical significance comparing Iso to indicated groups is shown (*** $P < 0.001$; ** $P < 0.01$). (B), the MEK inhibitor PD098059 (PD) did not have a significant (ns, non significant) effect on β -AR induced cytokine expression. (C), p38 mediates inflammatory gene expression during β -AR activation in vivo. 129S1/SvImJ mice were pretreated with the p38 inhibitor SB or vehicle followed by CL treatment (10 nmol) or H₂O for 3 hr and EWAT was analyzed for mRNA expression. Statistical significance for CL treatment within groups is noted (* $P < 0.01$, *** $P < 0.001$).