INTRODUCTION

Obesity often leads to increased systemic inflammation which is now thought to play a causative role in the development of atherosclerotic disease and insulin resistance. This inflammatory response originates within large adipose tissue depots and is initiated by classically activated macrophages that infiltrate the tissue from the circulation. The large number of macrophages resides in obese adipose tissue lead to significant increases in interleukin-6 (IL-6) and tumor necrosis factor-α (TNFα) secretion; achieving levels sufficient to elevate circulating plasma concentrations. These cytokines activate potent signals to initiate lipolysis, to release free fatty acids from triacylglycerol stores and contribute to hyperlipidemia in obese individuals. Obese adipose tissue responds to normal β-adrenergic and glucagon stimuli to recover from negative energy balance by inducing lipolysis. However, it is not clear what quantitative influence additional lipolytic stimulation by IL-6 and TNFα has on normal β-adrenergic activity. Although, β-adrenergic and cytokine signaling activate separate pathways for lipolytic activation, it is undefined if the effects of multiple signaling events on lipolysis are additive or coincident. To clarify this issue, we measured lipolytic activity in 3T3-L1-derived adipocytes stimulated by a β-adrenergic agonist (isoproterenol), IL-6 or TNFα individually and in combinations as co- and tri-stimulation. Treatment of adipocytes with isoproterenol and either IL-6 or TNFα as co-stimulants increased lipolytic activation by approximately the sum of the individual ligands suggesting contributions from two independent pathways. Co-stimulation with IL-6 and TNFα provided slightly more than an additive response indicating signaling contributions from independent and common pathways. Tri-stimulation resulted in the largest level of lipolytic activation with a value approximately to adding isoproterenol stimulation to a combined treatment of IL-6 and TNFα. The additive nature of cytokine signaling to β-adrenergic activity suggests its therapeutic inhibition will prevent excessive lipolysis, yet minimally interfere with maintaining normal responses to varying energy demands.

Additive Effects of B-Adrenergic and Cytokine Signaling on Lipolytic Activation

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Abstract

Obesity often leads to increased systemic inflammation which is now thought to play a causative role in the development of atherosclerotic disease and insulin resistance. This inflammatory response originates within large adipose tissue depots and is initiated by classically activated macrophages that infiltrate the tissue from the circulation. The large number of macrophages resides in obese adipose tissue lead to significant increases in interleukin-6 (IL-6) and tumor necrosis factor-α (TNFα) secretion; achieving levels sufficient to elevate circulating plasma concentrations. These cytokines activate potent signals to initiate lipolysis, to release free fatty acids from triacylglycerol stores and contribute to hyperlipidemia in obese individuals. Obese adipose tissue responds to normal β-adrenergic and glucagon stimuli to recover from negative energy balance by inducing lipolysis. However, it is not clear what quantitative influence additional lipolytic stimulation by IL-6 and TNFα has on normal β-adrenergic activity. Although, β-adrenergic and cytokine signaling activate separate pathways for lipolytic activation, it is undefined if the effects of multiple signaling events on lipolysis are additive or coincident. To clarify this issue, we measured lipolytic activity in 3T3-L1-derived adipocytes stimulated by a β-adrenergic agonist (isoproterenol), IL-6 or TNFα individually and in combinations as co- and tri-stimulation. Treatment of adipocytes with isoproterenol and either IL-6 or TNFα as co-stimulants increased lipolytic activation by approximately the sum of the individual ligands suggesting contributions from two independent pathways. Co-stimulation with IL-6 and TNFα provided slightly more than an additive response indicating signaling contributions from independent and common pathways. Tri-stimulation resulted in the largest level of lipolytic activation with a value approximately to adding isoproterenol stimulation to a combined treatment of IL-6 and TNFα. The additive nature of cytokine signaling to β-adrenergic activity suggests its therapeutic inhibition will prevent excessive lipolysis, yet minimally interfere with maintaining normal responses to varying energy demands.

signals for stimulation of lipolysis [10-13]. Increased lipolytic activity in obese adipose tissue increases free fatty acid (FFA) flux into the circulation. From a lipocentric view, elevated plasma levels of non-esterified fatty acids (NEFA) leads to increased amounts of atherogenic lipoproteins in the circulation resulting in unmanaged hyperlipidemia [14], often accompanied by reduced systemic insulin responsiveness [15,16].

The metabolic actions of IL-6 are diverse. Determination of which actions take precedence is largely dictated by tissue and metabolic context [17]. For example, IL-6 is an important mediator of the acute phase response which includes hepatic effects to increase glucose output and elevate CRP levels [17, 18]. IL-6 secretion is also increased as a result of exercise [19, 20], which in turn increases glucose oxidation [21] and insulin sensitivity [22] in skeletal muscle. IL-6 infusion in humans increased circulating FFA levels [11,23], and when adipose tissue...
or isolated adipocytes were treated with IL-6, lipolytic activity was increased [17]. In adipocytes, IL-6 binds to a cell surface heterodimer composed of IL-6 receptor and gp130 [24,25] and activates two intracellular signaling pathways, Janus kinase/ Signal Transducer and Activator of Transcription (JAK/STAT) and p44/42 Mitogen-activated protein kinase (MAPK) [17,26].

TNFα is a potent metabolic effector that, in adipocytes, signals primarily through TNFα receptor-1 [27]. Intracellular signaling in adipocytes is mediated by p44/42 MAPK and Jun N-terminal kinase (JNK) [12,28]. Once activated, these pathways induce phosphorylation of perilipin to recruit hormone sensitive lipase for triacylglycerol hydrolysis and release of FFA, and to downregulate perilipin expression [29,30]. Perilipin is a phosphoprotein that coats intracellular lipid droplets in adipocytes to maintain minimal lipolytic activity. Phosphorylation of perilipin serves a dual purpose: to release bound CGI-58 to activate Adipose Triglyceride Lipase and to relocate away from the lipid droplet permitting catalytic access for activated lipases [31]. However, signaling the sequence of events leading to lipolytic activation through TNFα is not the normal physiologic response to increased energy demands requiring release of fatty acid fuel stores from adipocytes. Normal physiologic activation of lipolysis by β-adrenergic and glucagon signaling during periods of increased systemic energy demands is mediated through heterotrimeric G-protein activation followed by increased intracellular cAMP and protein kinase-A (PKA) activation. Obese adipose tissue is subject to normal β-adrenergic and glucagon stimuli to regulate energy balance; however, this tissue is also subject to additional lipolytic stimuli by IL-6 and TNFα. With the presence of multiple pathways for lipolytic activation, i.e. the normal endocrine/ neural pathway and cytokine-mediated pathways, it is unclear if the effects of multiple signaling events on lipolysis are additive or coincident; that is, do IL-6 and TNFα stimulate lipolytic activities that are in excess of that provided by maximal β-adrenergic activation or do the pathways activated by these cytokines merge into the downstream β-adrenergic pathway and are unable to add significantly beyond maximal β-adrenergic activation. To address this question, we have measured lipolytic activity in 3T3-L1-derived adipocytes activated by a β-adrenergic agonist, IL-6 or TNFα individually and in combination as co- and tri-stimulation.

RESULTS AND DISCUSSION

Our primary objective for this study was to determine if cytokine stimulation (IL-6 or TNFα) heightens lipolytic activity (as measured by glycerol release) over and above what is stimulated by normal β-adrenergic signaling. To obtain this quantitative evaluation, we first incubated mature 3T3-L1-derived adipocytes with varying concentrations of either isoproterenol (a β-adrenergic agonist), IL-6 or TNFα individually in order to determine the concentration of ligand that provided maximal lipolytic stimulation. This will ensure that individual ligands will be used at concentrations that maximally activate their respective signaling pathways in co- and tri-stimulation experiments. From our titration study, we have determined that maximal lipolytic stimulation for each ligand is achieved with the following concentrations: 1 µM for isoproterenol, 2 nM for IL-6 and 0.58 nM for TNFα (data not shown).

Co- and tri-stimulation experiments were performed using ligand concentrations determined above. When adipocytes were incubated with individual ligands, different levels of lipolytic activation were noted with IL-6 < isoproterenol < TNFα (Figure 1, compare bar 2 < bar 1 < bar 5). These differences are likely due to activation of different signaling pathways which have varying quantitative effects on lipolytic activation. Co-stimulation of adipocytes with IL-6 and TNFα resulted in lipolytic activity that was greater than that stimulated by individual ligands (Figure 1, compare bar 3 with bars 1 and 2) suggesting parallel activation of different signaling pathways that merge into downstream lipolytic activation. The level of increased lipolytic activation from isoproterenol and IL-6 co-stimulation is approximately the sum of the individual ligands suggesting an additive effect from contributions from two independent pathways, likely cAMP/PKA and p44/42-JAK/STAT, respectively. When isoproterenol and TNFα were combined, again an additive effect on lipolytic activation was seen (Figure 1, compare bar 6 with bars 4 and 5), similarly suggesting summing the effects of two separate signaling pathways, cAMP/PKA and p44/42-JNK, respectively.

In vivo, obese adipose tissues express both IL-6 and TNFα when inflamed and susceptible to concurrent stimulation by both cytokines. To determine the effects of dual cytokine stimulation on lipolysis, adipocytes were incubated with IL-6 and TNFα.
β-adrenergic, epinephrine and norepinephrine, stimulation of adipose tissue due to stress responses or negative energy balance and this stimulation signals through the heterotrimeric G-protein, adenylyl cyclase, cAMP, PKA network. In addition to this signaling, cytokines produced in inflamed obese adipose tissue also activate additional pathways that make a cumulative addition to the normal lipolytic response. Evidence provided here suggests that IL-6- and TNFα-activated pathways in adipose contribute to increased lipolysis through both independent and common signaling pathways (Figure 2). In considering therapeutic options for obese individuals, maintaining normal β-adrenergic signaling is vital to manage routine changes in energy balance that occur due to cyclical variations in physical activity. However, the contributions of IL-6 and TNFα to increased lipolytic activity being additive to normal β-adrenergic stimulation indicates that these pathways (p44/42, JAK/STAT and JNK) represent excellent therapeutic targets for inhibition that will prevent excessive lipolysis, yet minimally interfere with maintaining normal responses to varying energy demands.

CONCLUSIONS

The amount of lipolytic activation from triple stimulation far surpassed that of normal β-adrenergic stimulation alone and provides mechanistic evidence for the cause of hyperlipidemia in obese individuals. Under normal circumstances in lean individuals, β-adrenergic signaling is activated during fasting and exercise to mobilize fatty acids from adipose tissue and compensate for negative systemic energy balance. Once energy balance has returned to homeostasis, β-adrenergic stimulation is inactivated and release of fatty acids is halted to prevent excessive plasma lipid levels. Obese individuals are also subject to normal β-adrenergic, epinephrine and norepinephrine, stimulation of adipose tissue due to stress responses or negative energy balance and this stimulation signals through the heterotrimeric G-protein, adenylyl cyclase, cAMP, PKA network. In addition to this signaling, cytokines produced in inflamed obese adipose tissue also activate additional pathways that make a cumulative addition to the normal lipolytic response. Evidence provided here suggests that IL-6- and TNFα-activated pathways in adipose contribute to increased lipolysis through both independent and common signaling pathways (Figure 2). In considering therapeutic options for obese individuals, maintaining normal β-adrenergic signaling is vital to manage routine changes in energy balance that occur due to cyclical variations in physical activity. However, the contributions of IL-6 and TNFα to increased lipolytic activity being additive to normal β-adrenergic stimulation indicates that these pathways (p44/42, JAK/STAT and JNK) represent excellent therapeutic targets for inhibition that will prevent excessive lipolysis, yet minimally interfere with maintaining normal responses to varying energy demands.

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REFERENCES


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