계명의대학술지
Keimyung Medical Journal

Original Article

pISSN 2092-8335 · eISSN 2733-5380
Keimyung Med J 2021;40(1):1-8
https://doi.org/10.46308/kmj.2021.00108

Received: May 21, 2021
Revised: June 4, 2021
Accepted: June 4, 2021

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Interleukin 1β Up-Regulates mRNA Expression of Inducible Nitric Oxide Synthase in 3T3-L1 Preadipocytes: Role of JAKs/STATs, PKCs, and Src

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Recent evidence suggests obesity as a low or systemic chronic inflammation. (Pre) adipocytes in the adipose tissue (AT) express and secrete a variety of cytokines and adipokines. Inducible nitric oxide synthase (iNOS) is an inflammatory enzyme involved in the production of NO. Until now, the inducer(s) of iNOS expression in (pre)adipocytes remains unclear. In this study, we investigated the effects of proinflammatory cytokines [interleukin-1β (IL-1β), IL-10, IL-12, interferon-γ (IFN-γ)], adipokines [retinol-binding protein 4 (RBP4), adiponectin, leptin, and resistin], and lipopolysaccharide (LPS), a bacterial cell wall component, on the expression of iNOS in 3T3-L1 preadipocytes. Notably, treatment with IL-1β at 20 ng/mL for 4 h markedly increased iNOS mRNA expression in 3T3-L1 preadipocytes, but that with IL-10 (10 ng/mL), IL-12 (5 ng/mL), IFN-γ (10 ng/mL), RBP4 (5 ug/mL), adiponectin (100 ng/mL), leptin (100 ng/mL), and resistin (100 ng/mL), and LPS (1 ug/mL) for 4 h had little or no effect on it. Results of dose-response and time-course experiments confirmed the ability of IL-1β at 20 ng/mL for 4 h to maximally induce iNOS mRNA expression in 3T3-L1 preadipocytes. Importantly, pharmacological inhibition studies demonstrated that treatment with AG490 [an inhibitor of Janus-activated kinases (JAKs) and signal transducer and activator of transcription proteins (STATs)], GO6976 (an inhibitor of PKCs), or PP1 (an Src kinase inhibitor) suppressed IL-1β-induced iNOS mRNA expression in 3T3-L1 preadipocytes, pointing out the involvement of JAKs/STATs, PKCs, and Src in the process. This work advocates that IL-1β is a major and strong inducer of iNOS expression in 3T3-L1 preadipocytes.

Keywords: 3T3-L1 Cells, Apha-cyano-(3,4-dihydroxy)-N-benzylcinnamide, GO6976, Inducible nitric oxide synthase, Interleukin-1β

Introduction

Obesity is a major health concern, often deteriorating life expectancy and increasing risks of many human diseases, such as type 2 diabetes mellitus, cardiovascular diseases, hypertension, non-alcoholic fatty liver disease, osteoarthritis, and cancer [1]. It is documented that obesity is defined as an increase in body mass fat in the adipose tissue (AT) resulting from excessive preadipocyte differentiation in the human body [2,3]. However, there is recent evidence alternatively proposing obesity as a low or systemic chronic inflammation [4].

A large body of evidence illustrates that preadipocytes, adipocytes, and macrophages in the AT secrete an array of cytokines and adipokines, which are involved in anti- or pro-inflammatory roles [5]. A wealth of information also indicates that (pre)adipocytes are exposed to different endogenic (cytokines, adi-

pokines, free fatty acids) and exogenic [lipopolysaccharide (LPS), a bacterial cell wall component] stimuli [6-8], which may aggregate inflammation in the AT and thus obesity inflammation. It has been of interest demonstrated that tumor necrosis factor-a (TNF-α), a pro-inflammatory cytokine, stimulates (pre)adipocytes to express and secrete many inflammatory mediators and chemokines [9], thereby exacerbating inflammation and recruiting macrophages in the AT [10]. These results suggest that (pre)adipocytes or macrophages-derived other adipokines and/or cytokines also can stimulate (pre)adipocytes to express and secrete inflammatory mediators.

Inducible nitric oxide synthase (iNOS) is an inflammatory enzyme involved in the production of NO [11,12]. NO is generated from L-arginine and molecular oxygen by the action of three NOSs: the neuronal NOS (nNOS), endothelial NOS (eNOS), and iNOS [13,14]. At the physiological level, NO produced by eNOS and nNOS is an essential signaling molecule involved in vascular homeostasis, neurotransmission, and immune defense against infectious agents [15]. Of importance, there is a wealth of information demonstrating that the expression and activity of iNOS are abnormally increased in inflammation and cancer, and the resultant NO overproduction plays a causative role in these pathologies [16]. Notably, growing body of evidence further points out the role of iNOS in AT or obesity inflammation, as evidenced by that iNOS deficiency in ob/ob mice improved AT inflammation through lower AT macrophage infiltration and a down-regulation of proinflammatory and profibrogenic genes [17]. It also has been reported that the high amount of NO synthesized by iNOS acts with reactive oxidative species producing nitrosative stress, playing a key role in the impairment of adipocyte function and the development of obesity [18]. In characteristic, while eNOS and nNOS are constitutively expressed in endothelial and neuronal cells, iNOS expression is largely increased in many types of cells exposed to interferon-γ (IFN-γ), a cytokine or LPS [19,20]. Expression of iNOS is regulated at transcription and translation [21]. iNOS expression is also influenced by the expression and activity of multiple intracellular signaling proteins, including the family of Janus-activated kinases (JAKs) and signal transducer and activator of transcription proteins (STATs), nuclear factor-κB (NF-κB), mitogen-activated protein kinases (MAPKs), protein kinase Cs (PKCs), and Src non-receptor kinase [22,23]. Of interest, it has been previously shown that TNF-α is a strong inducer of iNOS in many types of cells, including (pre) adipocytes [24]. Supporting this, we also have recently demonstrated the ability of TNF-α to induce iNOS expression in 3T3-L1 (pre)adipocytes [25]. However, at present, the inducer(s) and its regulation of iNOS expression in (pre)adipocytes remain unclear.

In this study, we investigated the effects of a subset of cytokines [interleukin-1β (IL-1β), IL-10, IL-12, interferon-γ (IFN-γ)], adipokines [(retinol-binding protein 4 (RBP4), adiponectin, leptin, and resistin] and LPS on the expression of iNOS in 3T3-L1 preadipocytes. Strikingly, we have found that among the endogenic and exogenic stimuli tested, IL-1β strongly induces iNOS mRNA expression in 3T3-L1 preadipocytes. Our data also indicate that the cytokine-induced iNOS mRNA expression in 3T3-L1 preadipocytes is linked to the activities of JAKs/STATs, PKCs, and Src kinase.

Materials and methods

1. Materials

IL-1β was purchased from R&D Systems (Minneapolis, MN, USA). Benzyloxy carbonyl Leu Leu phenylalaninal Inhibitor (ZLLF-CHO, the chymotrypsin-like activity inhibitor), MG132 (a 26S proteasome inhibitor), SP600125 (an inhibitor of JNK-1/2), salubrinal (an inhibitor of eIF-2α dephosphorylation), and PP1 (an inhibitor of Src) were obtained from Calbiochem (La Jolla, CA, USA). SB203580 (an inhibitor of p38 MAPK), PD98059 (an inhibitor of ERK-1/2), Hispidin (an inhibitor of PKC-β), LY294002 (an inhibitor of PI3K/PKB), GF109203X (a pan-inhibitor of PKCs), H89 (an inhibitor of PKA), and GO6976 (a pan-inhibitor of PKCs) were purchased from Biomol (Plymouth, PA, USA). GO6983 (an inhibitor of PKC-α/β/γ/δ/ζ) was purchased from Promega (Madison, WI, USA). Antibodies of phospho (p)-STAT-3 and STAT-3 were obtained from Santa Cruz Biotechnology (Delaware, CA, USA). Antibodies of p-Src, Src, p-JNK-1/2, p-ERK-1/2, and IκB-α were bought from Cell Signaling Technology (Danvers, CO, USA). AG490 (an inhibitor of JAKs/STATs) and antibody of β-actin were purchased from Sigma (St. Louis, MO, USA). Antibodies against anti-rabbit or mouse secondary horseradish peroxidase and enhanced chemiluminescence (ECL) Western detection reagents were bought from Amersham Biosciences (Corston, Bath, UK). ECL reagent was bought from Advansta (Menlo Park, CA, USA).

2. Cell culture

3T3-L1 murine white preadipocytes (ATCC, Manassas, VA, USA) were grown in DMEM supplemented with 10% newborn calf serum (Gibco, Thermo Fisher, New Zealand) and

1% penicillin-streptomycin (Welgene, Daegu, Korea).

3. Reverse-transcription polymerase chain reaction (RT-PCR) analysis.

Total cellular RNA in the control or agents-treated 3T3-L1 preadipocytes at the designated time point was isolated with the RNAiso Plus (TaKaRa, Kusatsu, Shiga, Japan). Three micrograms of total RNA were reverse transcribed using a random hexadeoxynucleotide primer and reverse transcriptase. Single-stranded cDNA was amplified by PCR with the following primers: iNOS sense 5'-GACAAGCTGCATGTGACATC-3'; antisense 5'-GCTGGTAGGTTCCTGTTGTT-3'; β-actin sense 5'-TCATGAAGTGTGACGTTGACATCCGT-3'; antisense 5'-CCTAGAAGCATTTGCGGTGCACGATG-3'; Expression levels of β-actin mRNA expression were used as an internal control as well as the protein loading control.

4. Preparation of whole cell lysates (total cellular proteins)

At the designated time point, the control or agents-treated 3T3-L1 preadipocytes were washed twice with PBS and lyzed with a modified RIPA buffer [50 mM Tris-Cl (pH 7.4), 150 mM NaCl, 0.1% sodium dodecyl sulfate, 0.25% sodium deoxycholate, 1% Triton X-100, 1% Nonidet P-40, 1 mM EDTA, 1 mM EGTA, proteinase inhibitor cocktail (1x)]. The cell lysates were then collected and centrifuged at 13,000 rpm for 15 min at 4°C. The supernatant was saved, and its protein concentration was determined with Pierce BCA Protein Assay Kit (Thermo Scientific, Rockford, IL, USA).

5. Western blot analysis

Proteins (30 μg) were separated by SDS-PAGE (10%) and transferred onto a PVDF membrane (Millipore, Burlington, MA, USA). The membrane was washed with TBS (10 mM Tris, 150 mM NaCl) supplemented with 0.05% (vol/vol) Tween 20 (TBST) followed by blocking with TBST containing 5% (vol/vol) non-fat dried milk. The membrane was incubated overnight with respective antibody of p-STAT-3 (1:2,000), STAT-3 (1:2,000), Src (1:2,000), p-Src (1:2,000), p-JNK-1/2 (1:2,000), p-ERK-1/2 (1:2,000), IκB-α (1:2,000) or β-actin (1:10,000) at 4°C. The membrane was washed three times with TBST at room temperature (RT). The membrane was then exposed to a secondary antibody coupled to horseradish peroxidase for 2 h at RT. The membrane was washed three times with TBST at RT. Immunoreactivities were detected by ECL reagents. Equal protein loading was assessed by the expression level of β-actin protein.

Results

1. Treatment with IL-1β (20 ng/ml, 4 h) strongly and selectively induces high mRNA expression of iNOS in 3T3-L1 preadipocytes

Initially, we investigated the effects of pro-inflammatory cytokines (IL-1β, IL-10, IL-12, IFN-γ), adipokines (RBP4, adiponectin, leptin, and resistin) and LPS on regulation of iNOS expression in 3T3-L1 preadipocytes using RT-PCR analysis. Strikingly, as shown in Fig. 1A, treatment with IL-1β at 20 ng/mL for 4 h led to high mRNA expression of iNOS in 3T3-L1 preadipocytes. However, treatment with IL-10 (10 ng/mL), IL-12 (5 ng/mL), IFN-γ (10 ng/mL), RBP4 (5 μg/mL), adiponectin (100 ng/mL), leptin (100 ng/mL), resistin (100 ng/mL) or LPS (1 μg/mL) for 4 h had little or no effect on iNOS mRNA expression in 3T3-L1 preadipocytes (Fig. 1A, B). These results point out the selective ability of this pro-inflam-

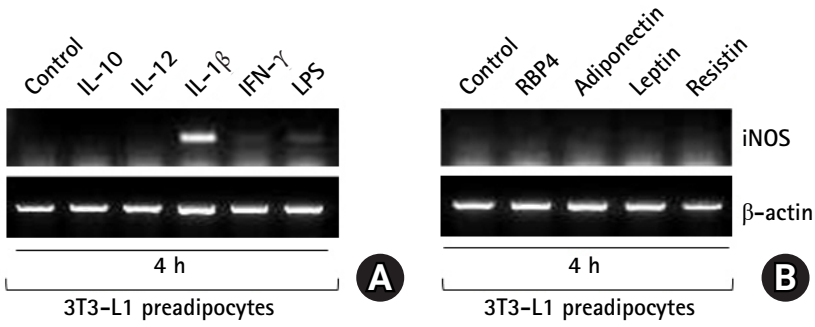

Fig. 1. Effects of different cytokines, adipokines and LPS on iNOS mRNA expression in 3T3-L1 preadipocytes.
(A) 3T3-L1 preadipocytes were treated without or with IL-1β (20 ng/mL), IL-10 (10 ng/mL), IL-12 (5 ng/mL), IFN-γ (10 ng/mL) or LPS (1 μg/mL) for 4 h. Total cellular RNA from the conditioned cells was prepared and analyzed by RT-PCR to measure mRNA expression levels of iNOS or β-actin. (B) 3T3-L1 preadipocytes were treated without or with RBP4 (5 μg/mL), adiponectin (100 ng/mL), leptin (100 ng/mL), and resistin (100 ng/mL) for 4 h. Total cellular RNA from the conditioned cells was prepared and analyzed by RT-PCR to measure mRNA expression levels of iNOS or β-actin.

matory cytokine to strongly up-regulate iNOS mRNA expression in 3T3-L1 preadipocytes. Because of high induction of iNOS mRNA expression, we chose IL-1β for further studies.

2. Treatment with IL-1β leads to a concentration- and time-dependent increase of iNOS mRNA expression in 3T3-L1 preadipocytes

We next examined the effect of IL-1β at different concentrations for 4 h on iNOS mRNA expression in 3T3-L1 preadipocytes. As shown in Fig. 2A, treatment with IL-1β at 1 or 5 ng/mL did not induce iNOS mRNA expression in 3T3-L1 preadipocytes, but that with IL-1β at 10 ng/mL led to a weak induction of iNOS mRNA expression in these cells. However, as anticipated, treatment with IL-1β at 20 ng/mL resulted in strong induction of iNOS mRNA expression in 3T3-L1 preadipocytes, illustrating the maximal iNOS induction by 20 ng/mL of IL-1β. Because of high induction of iNOS mRNA expression, we selected this 20 ng/mL of IL-1β for further studies. Kinetic studies were next carried out to know the time of iNOS mRNA expression in 3T3-L1 preadipocytes treated with IL-1β (20 ng/mL). Apparently, as shown in Fig. 2B, treatment with IL-1β led to a time-dependent increase of iNOS mRNA expression in 3T3-L1 preadipocytes. Vividly, maximal iNOS mRNA expression was seen at 4 h treatment with IL-1β. Notably, there was a sharp decline of iNOS transcripts in 3T3-L1 preadipocytes thereafter.

3. Treatment with IL-1β leads to the altered expression and phosphorylation levels of intracellular proteins in 3T3-L1 preadipocytes

Aforementioned, multiple transcription factors and signaling proteins, including JAKs, STATs, NF-κB, Src kinase, and MAPKs, participate in pro-inflammatory cytokine(s)-induced iNOS expression in many types of cells [22,23]. This led us to investigate whether IL-1β (20 ng/mL) treatment affects the expression and phosphorylation levels of ERK-1/2, JNK-1/2, inhibitory kappa B-α (IκB-α), STAT-3, and Src in 3T3-L1 preadipocytes over time. Notably, as shown in Fig. 3, compared with control, treatment with IL-1β led to a slight increase in the phosphorylation levels of ERK-1/2 in 3T3-L1 preadipocytes at the times tested. However, there was no detection of phosphorylated JNK-1/2 in 3T3-L1 preadipocytes treated with IL-1β at the times tested. Strikingly, treatment with IL-1β at 0.25, 0.5 or 1 h caused a big loss of IκB-α in 3T3-L1 preadipocytes. Moreover, IL-1β treatment at 1 or 2 h led to a slight increase in the phosphorylation and expression levels of STAT-3 and Src in 3T3-L1 preadipocytes. Control actin protein expression remained largely unchanged under these experimental conditions.

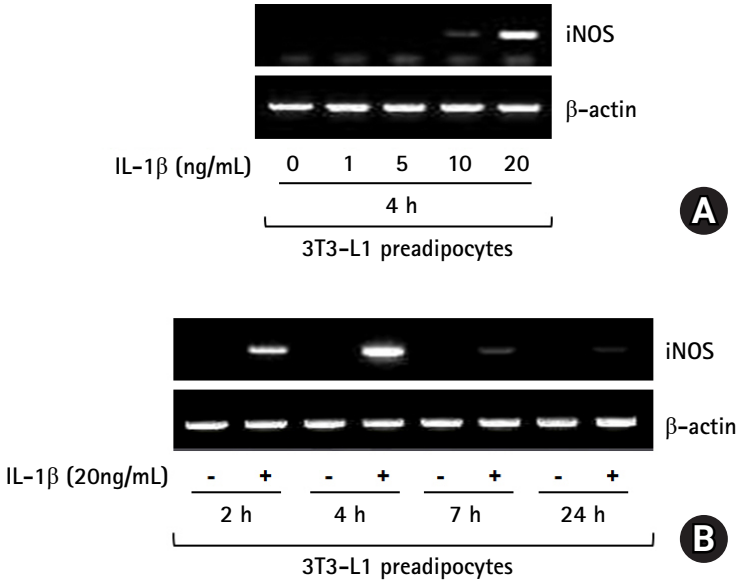

Fig. 2. Concentration and time-dependent effects of IL-1β on iNOS mRNA expression in 3T3-L1 preadipocytes.
(A) 3T3-L1 preadipocytes were treated without or with IL-1β at the designated concentrations for 4 h. Total cellular RNA from the conditioned cells was prepared and analyzed by RT-PCR to measure mRNA expression levels of iNOS or β-actin. (B) 3T3-L1 preadipocytes were treated with IL-1β (20 ng/mL) at the designated time points. At each time point, total cellular RNA from the conditioned cells was prepared and analyzed by RT-PCR to measure mRNA expression levels of iNOS or β-actin.

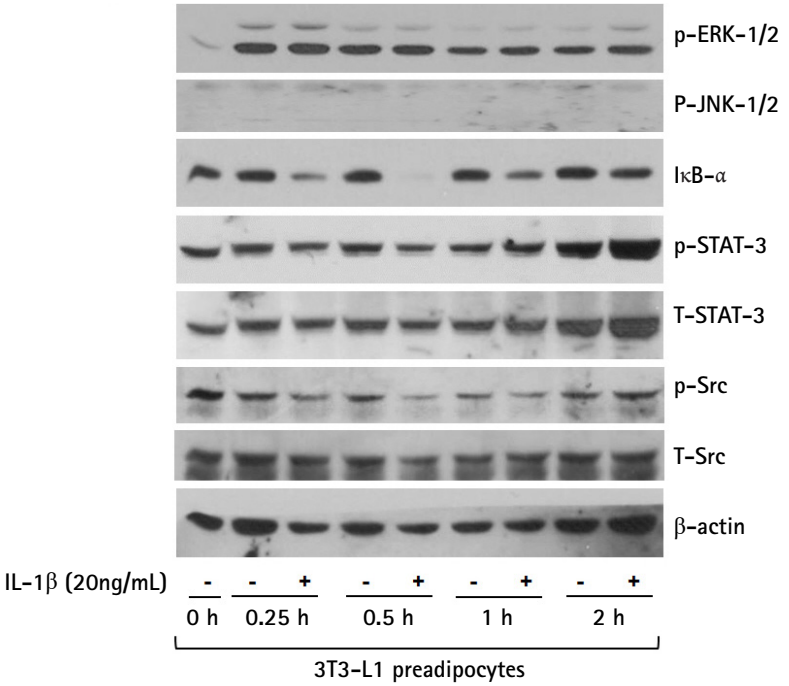

Fig. 3. Effects of IL-1β on expression and phosphorylation of transcription factors and signaling proteins in 3T3-L1 preadipocytes over time.
3T3-L1 preadipocytes were treated without or with IL-1β (20 ng/mL) for the designated time points. At each time point, whole cell lysates from the conditioned cells were prepared and subjected to Western blot analysis to measure protein expression and phosphorylation levels of ERK-1/2, JNK-1/2, IκB-α, STAT-3, Src or β-actin. p-ERK-1/2, phosphorylated ERK-1/2; p-JNK-1/2, phosphorylated JNK-1/2; p-STAT-3, phosphorylated STAT-3; p-Src, phosphorylated Src.

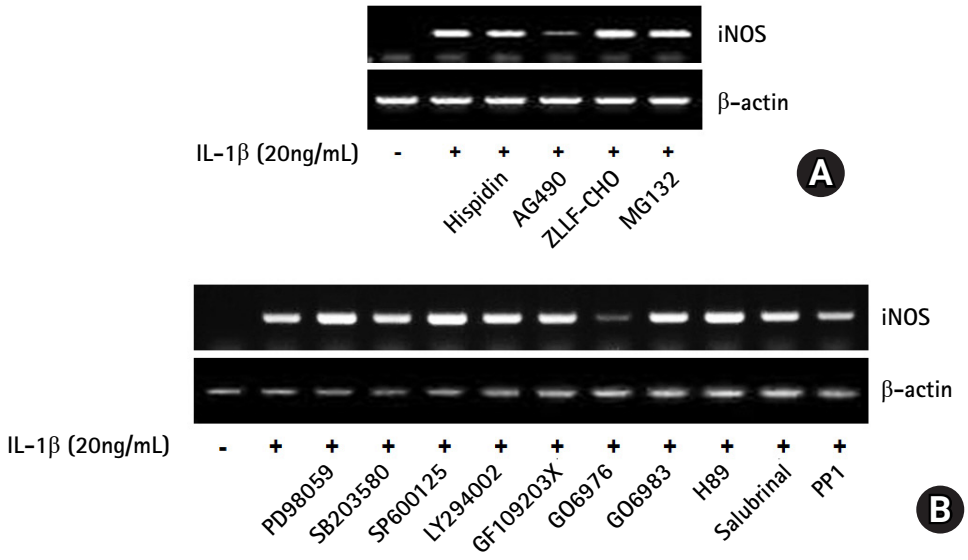

Fig. 4. Effects of IL-1β and/or various pharmacological inhibitors on iNOS mRNA expression in 3T3-L1 preadipocytes.
(A) 3T3-L1 preadipocytes were treated without or with IL-1β (20 ng/mL) in the absence or presence of Hispidin (an inhibitor of PKC-β, 10 μM), AG490 (an inhibitor of JAKs/STATs, 100 μM), ZLLF-CHO (the chymotrypsin-like activity inhibitor, 10 μM) or MG132 (an inhibitor of 26S proteasome, 5 mM) for 4 h. Total cellular RNA from the conditioned cells was prepared and analyzed by RT-PCR to measure mRNA expression levels of iNOS or β-actin. (B) 3T3-L1 preadipocytes were treated without or with IL-1β (20 ng/mL) in the absence or presence of PD98059 (an inhibitor of ERK-1/2, 50 μM), SB203580 (an inhibitor of p38 MAPK, 25 μM), SP600125 (an inhibitor of JNK-1/2, 25 μM), LY294002 (an inhibitor of PI3K/PKB, 25 μM), GF109203X (a pan-inhibitor of PKCs, 10 μM), GO6976 (a pan-inhibitor of PKCs), GO6983 (an inhibitor of PKCα/β/γ/δ/ζ, 10 μM), H89 (an PKA inhibitor, 10 μM), salubrinal (an inhibitor of eIF-2α, 10 μM), and PP1 (an inhibitor of Src, 10 μM) for 4 h. Total cellular RNA from the conditioned cells was prepared and analyzed by RT-PCR to measure mRNA expression levels of iNOS or β-actin.

4. Treatment with AG490 (an inhibitor of JAKs/STATs), GO6976 (an inhibitor of PKCs) or PP1 (a Src kinase inhibitor) suppressed IL-1β-induced iNOS mRNA expression in 3T3-L1 preadipocytes

Using pharmacological inhibitors, we next sought to explore which transcription factors and signaling proteins mediate IL-1β-induced iNOS mRNA expression in 3T3-L1 preadipocytes. Pharmacological inhibitors used herein included Hispidin, AG490, ZLLF-CHO, MG132, PD98059, SB203580, SP600125, LY294002, GF109203X, GO6976, GO6983, H89, salubrinal, and PP1. Of note, as shown in Fig. 4A, while treatment with Hispidin (10 μM), ZLLF-CHO (10 μM) or MG132 (5 mM) had no effect on IL-1β-induced iNOS mRNA expression in 3T3-L1 preadipocytes, that with AG490 (100 μM) strongly suppressed it. Furthermore, as shown in Fig. 4B, treatment with GO6976 (10 μM) strongly attenuated IL-1β-induced iNOS mRNA expression in 3T3-L1 preadipocytes, and that with PP1 (10 μM) also partially interfered with it. However, treatment with PD98059 (50 μM), SB203580 (25 μM), SP600125 (25 μM), LY294002 (25 μM), GF109203X (25 μM), GO6983 (10 μM), H89 (10 μM) or salubrinal (10 μM) had no effect on IL-1β-induced iNOS mRNA expression in 3T3-L1 preadipocytes.

Discussion

Recent evidence strongly indicates that a low or systemic inflammation contributes to the development of obesity. (Pre) adipocytes express and secrete an array of cytokines and adipokines, collectively named as adipocytokines or adipokines. iNOS is an inflammatory enzyme that is involved in production of NO. Until now, the inducer(s) and its regulation of iNOS expression in (pre)adipocytes remain unclear. In this study, we demonstrate that IL-1β, a pro-inflammatory cytokine, largely induces iNOS mRNA expression in 3T3-L1 preadipocytes, which appears to be dependent on the activities of JAKs, STATs, PKCs, and Src kinase.

Aforementioned, we and others have previously shown that TNF-α, another pro-inflammatory cytokine, up-regulates the expression of iNOS in (pre)adipocytes [24,25]. Given that (pre)adipocytes express and secrete not only TNF-α but also other pro-inflammatory cytokines, and they are exposed to many endogenic and exogenic stimuli, we herein have investigated the effects of endogenic (IL-1β, IL-10, IL-12, IFN-γ) and exogenic (LPS) stimuli on the expression of iNOS in 3T3-L1 preadipocytes. Notably, the present study has demonstrated the ability of IL-1β to strongly and selectively induce iNOS mRNA expression in 3T3-L1 preadipocytes, based on the facts that IL-10, IL-12, IFN-γ or LPS has no inductive effect on it. In general, pro-inflammatory cytokines and LPS exert their biological activities, including gene expression, via direct binding or interaction with their cognate receptors expressed on the surface of cells [26]. Thus, considering the IL-1β's ability to induce iNOS mRNA expression in 3T3-L1 preadipocytes herein, it is likely that 3T3-L1 preadipocytes express functional IL-1β receptor, but they do not express IL-10, IL-12, IFN-γ or LPS receptor at the cell surface. It is documented that upon the exposure of differentiation cocktails 3T3-L1 preadipocytes become mature 3T3-L1 adipocytes filled with many lipid droplets through differentiation process, also named adipogenesis [27], and that mature 3T3-L1 adipocytes express and secrete overproduction of pro-inflammatory cytokines, and these cytokines stimulate (pre)adipocytes in autocrine and paracrine manners [28], thereby aggregating inflammation in the AT. In line of this, it will be interesting to examine, in future, whether differentiated 3T3-L1 adipocytes secrete and express not only IL-1β and IL-1β receptor but also IL-12 and IFN-γ and their receptors at the cell surface, and they are functional in inducing the expression of iNOS (and other inflammatory enzymes or mediators). Distinctly, in this study, we have known no inducibility of iNOS mRNA expression by 4 different adipokines (RBP4, adiponectin, leptin, resistin) tested in 3T3-L1 preadipocytes. These results point out that 3T3-L1 preadipocytes may not express RBP4, adiponectin, leptin or resistin receptor at the cell surface. Given that mature 3T3-L1 adipocytes express and secrete high levels of adipokines including RBP4, adiponectin, leptin and resistin [29], and these adipokines regulate (pre)adipocytes in autocrine and paracrine manners [30], it will be thus interesting to see, in future, whether differentiated 3T3-L1 adipocytes express these adipokine receptors and they are functional in inducing the expression of iNOS (and other inflammatory enzymes or mediators).

Mounting evidence demonstrates that iNOS expression is regulated at transcription [13,14,31]. Previous studies have shown that pro-inflammatory cytokine(s)-induced iNOS transcription is largely dependent on activities of transcription factors, including NF-κB, which acts on its cognate cis-acting element(s) present in the iNOS promoter [32-34]. Indeed, there is escalating evidence strongly supporting that activation of NF-κB is crucial for the cytokine-induced iNOS expression in many types of cells [35]. In resting cells, NF-κB is inactive because of its cytoplasmic retention by a physical interaction with IκB-α, a cytoplasmic inhibitory protein of

NF-κB [36]. When cells are exposed to extracellular stimuli, IκB-α is rapidly phosphorylated, poly-ubiquitinated, and degraded by 26S proteasome pathway, thereby leading to activation of NF-κB [37]. The present study has clearly demonstrated the ability of IL-1β to rapidly induce activation of NF-κB in 3T3-L1 preadipocytes, which is supported by that IL-1β induces a rapid and large loss of IκB-α in these cells. However, we herein have shown that MG132, a 26S proteasome pathway inhibitor, does not suppress IL-1β-induced iNOS mRNA expression in 3T3-L1 preadipocytes. These results point out that although IL-1β rapidly triggers NF-κB activation in 3T3-L1 preadipocytes, the IL-1β-induced NF-κB activation does not play a pivotal role in the cytokine-induced iNOS mRNA expression in 3T3-L1 preadipocytes. These results may thus indicate presence of other transcription factors responsible for the IL-1β-induced iNOS mRNA expression in 3T3-L1 preadipocytes. Aforementioned, expression of iNOS is also regulated by the expression and activity of other transcription factors and signaling proteins, including the family of JAKs and STATs, MAPKs, PKCs, PI3K/PKB, and Src [14,15]. In the current study, we have shown that IL-1β increases the phosphorylation and expression levels of STAT-3 in 3T3-L1 preadipocytes, and AG490, an inhibitor of JAKs/STATs, greatly suppresses IL-1β-induced iNOS mRNA expression in these cells, addressing that activation of the family of JAKs/STATs appears to play a partial role in the IL-1β-induced iNOS mRNA expression in 3T3-L1 preadipocytes. Furthermore, considering the present findings that IL-1β increases Src phosphorylation and expression in 3T3-L1 preadipocytes, and PP1, a Src inhibitor, substantially blocks the IL-1β-induced iNOS mRNA expression in these cells, it is likely that Src activation also contributes to the cytokine-induced iNOS mRNA expression in 3T3-L1 preadipocytes.

In summary, we demonstrate that IL-1β induces high iNOS mRNA expression in 3T3-L1 mouse preadipocytes, and this cytokine-induced iNOS mRNA expression in these cells largely depends on the activities JAKs/STATs, PKCs, and Src kinase. Although there are still important issues that remain to be resolved, including IL-1β's ability to induce iNOS expression in mature 3T3-L1 adipocytes and human (pre)adipocytes, our present findings show IL-1β is a strong inducer of iNOS expression in 3T3-L1 preadipocytes..

Conflict of interest

All authors declare no conflicts-of-interest related to this article.

Acknowledgements

We deeply thank Dr. Anil Kumar Yadav for his technical assistance in preparing this manuscript.

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