FEBS Letters 583 (2009) 3045–3049

FEBS Letters

journal homepage: www.FEBSLetters.org

Chronic TNFα and cAMP pre-treatment of human adipocytes alter HSL, ATGL and perilipin to regulate basal and stimulated lipolysis

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ARTICLE INFO

Article history:
Received 4 June 2009
Revised 31 July 2009
Accepted 11 August 2009
Available online 18 August 2009

Edited by Laszlo Nagy

Keywords:
Hormone-sensitive lipase
Adipose triglyceride lipase
Lipolysis
TNFα
Forskolin
Protein kinase A

ABSTRACT

We examined the effects of chronic TNFα and dibutyryl-cAMP (Db-cAMP) pre-treatment on the lipolytic machinery of human hMADS adipocytes. TNFα decreased adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) protein content and triglycerides (TG)-hydrolase activity but increased basal lipolysis due to a marked reduction in perilipin (PLIN) protein content. Conversely, Db-cAMP increased ATGL and HSL protein content but prevented PLIN phosphorylation, the net result being accentuated basal lipolysis. In forskolin-stimulated conditions, TNFα and Db-cAMP pre-treatment decreased stimulated TG-hydrolase activity and impaired PLIN phosphorylation. Together, this resulted in a severely attenuated response to forskolin-stimulated lipolysis.

1. Introduction

The regulation of human adipose tissue lipolysis is a complex multi-factorial process. Alterations of lipolysis and lipase expression have been shown in obesity and insulin resistance [1–3]. Lipolysis is governed by adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL). HSL displays in vitro affinity for both triglyceride (TG) and diglyceride (DG) molecules [4,5] while the recently discovered ATGL exerts affinity for TG only [6]. In fact, a body of evidence has emerged suggesting that despite their common capacity to hydrolyze TG, ATGL and HSL act sequentially [7–9]. To fully exert its action on lipid breakdown, ATGL requires the coactivator CGI-58, which in itself is devoid of TG-hydrolase activity [10]. Lipases access to stored lipids is dependent upon perilipin (PLIN), a member of the Perilipin, Adipophilin, TIP-47 (PAT) protein family which decorates lipid droplets (LD) of the adipocyte [11].

Acute regulation of the lipolytic machinery in fat cells occurs at the post-translational level. In human adipocytes, catecholamines and natriuretic peptides induce the activation of protein kinase A (PKA) and G, respectively [12]. The protein kinases phosphorylate HSL and PLIN [13,14] leading to CGI-58 release [15], HSL translocation [16,17] and LD fragmentation [18]. The prominent role of ATGL in PKA-stimulated lipolysis has recently been shown in murine [9,19] and human adipocytes [7] and specifically attributed to the phosphorylation of PLIN on serine 517 [20].

Determinants of long term regulation of lipolysis and lipase action remain largely unknown. Sustained activation of the sympathetic nervous system may lead to desensitization of catecholamine-stimulated lipolysis [21,22]. Moreover, TNFα has been shown to modulate human fat cell lipolysis [23,24]. Herein, we used a unique human white adipocyte cell model, termed hMADS adipocytes [7] to examine adaptations of the lipolytic machinery to prolonged TNFα exposure and sustained PKA activation with dibutyryl-cyclic AMP (Db-cAMP) [25]. The specific HSL inhibitor 4-isopropyl-3-methyl-2-[1-(3-(*S*)-methyl-piperidin-1-yl)-methanoyl]-2*H*-isoxazol-5-one

Abbreviations: ACS, acyl-CoA synthase; ATGL, adipose triglyceride lipase; Bay, 4-isopropyl-3-methyl-2-[1-(3-(*S*)-methyl-piperidin-1-yl)-methanoyl]-2*H*-isoxazol-5-one; DG, diglycerides; Db-cAMP, dibutyryl-cyclic AMP; FK, forskolin; hMADS cells, human adipose tissue derived-multipotent stem cells; HSL, hormone-sensitive lipase; JNK, c-*jun* NH_2 terminal kinase; LD, lipid droplet; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor κB; NPRA, natriuretic peptide receptor A; OA, oleic acid; PAT, Perilipin, adipophilin, TIP-47; PDE3B, phosphodiesterase 3B; PKA, protein kinase A; PLIN, perilipin; TG, triglycerides

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0014-5793/$36.00
doi:10.1016/j.febslet.2009.08.019

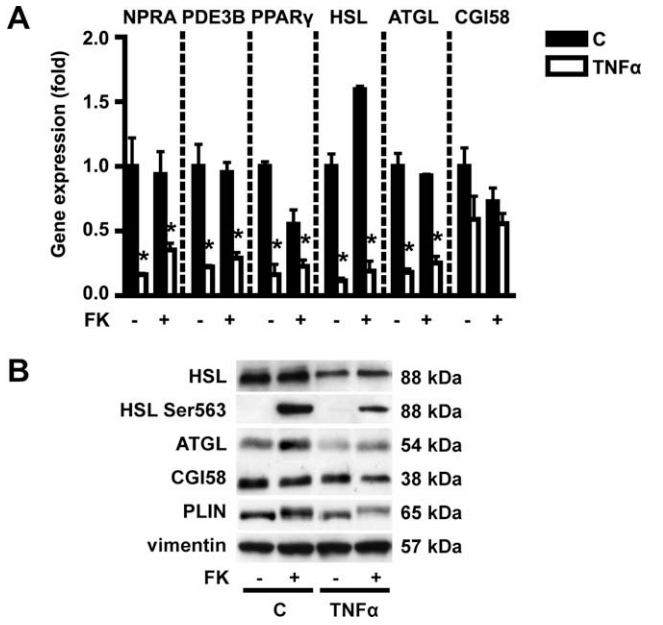

Fig. 1. Gene and protein expression of hMADS lipolytic machinery after chronic treatment with TNFα. Cells were pre-treated for 72 h with TNFα (100 ng/mL) from Day 12 to Day 15. On Day 15, TNFα was removed and cells were acutely treated for 3 h with or without FK (1 μM) and harvested for gene expression and Western blot analysis. (A) Gene expression of NPRA, PDE3B, PPARγ, HSL, ATGL and CGI-58 assessed by real-time RT-PCR and normalized with 18S rRNA levels. (B) Western blots of HSL, HSL Ser^{563}, ATGL, CGI-58 and PLIN, normalized to vimentin. The data are presented as means ± S.E.M. $N = 3–6$. * Significantly different from control condition (C).

(Bay) was used to discriminate between HSL and ATGL specific actions [2]. Consequences on the lipolytic machinery protein content, TG-hydrolase activity, and lipolysis were examined in basal and forskolin (FK)-stimulated states.

2. Materials and methods

2.1. Cell culture

Human adipose tissue derived-multipotent stem cells (hMADS cells) were cultured as previously described [7]. Experiments were held on Days 12–15 of differentiation. Pharmacological treatments of cells were both chronic and acute. Chronic pre-treatment with 100 ng/mL TNFα (72 h) or 1 mM Db-cAMP (48 h) was administered and removed prior to acute manipulations. Acute treatment (3 h) with 1 μM FK and/or 10 μM specific HSL inhibitor Bay [2] was added prior to harvesting cells or during functional measurements.

2.2. Determination of mRNA levels

Total RNA was extracted using the RNeasy total RNA mini kit (Qiagen) and processed as previously described [7].

2.3. Immunoblotting

Western blots and revelation were performed as described [7]. Total hMADS cell homogenates were prepared in extraction buffer (10 mM Tris HCl – pH 7.4, 0.25 M sucrose, 1 mM EDTA, 1 mM DTT) or Laemmli sample buffer. Primary antibodies used were anti-hHSL (1:12 000, gift from Dr. Cecilia Holm, Lund, Sweden), anti-hHSL-

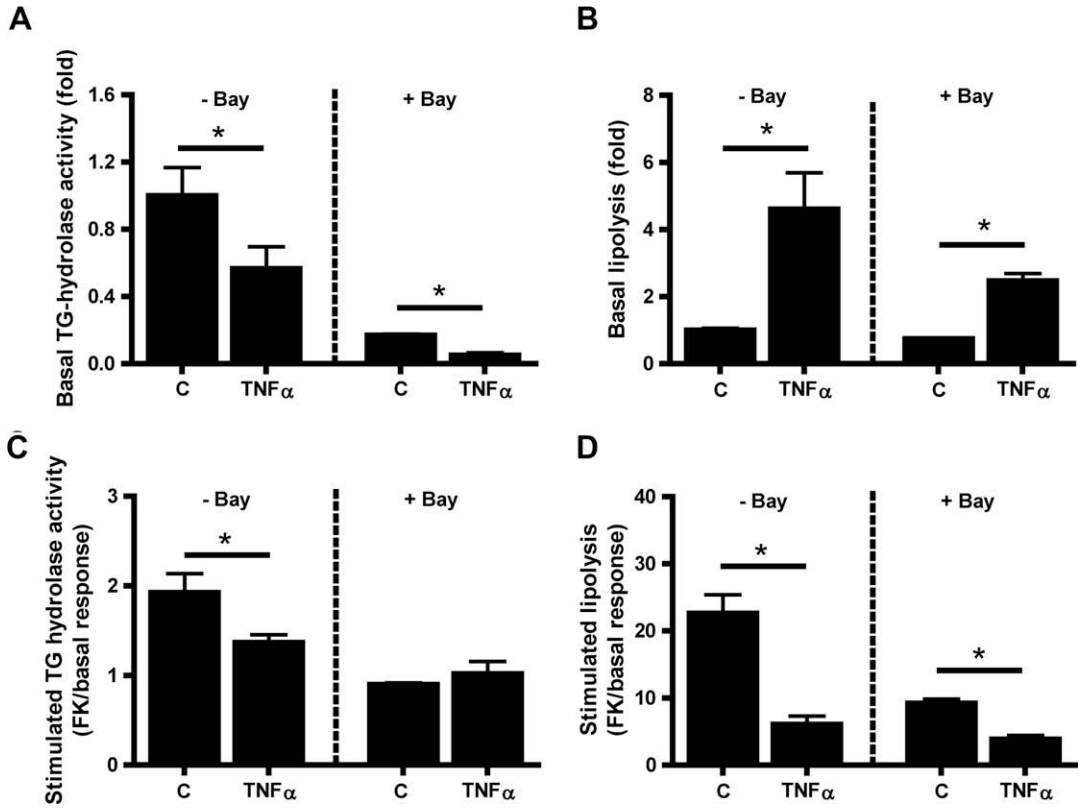

Fig. 2. TG-hydrolase activity and lipolysis of hMADS cells after chronic treatment with TNFα. Cells were pre-treated for 72 h with TNFα (100 ng/mL) Day 12 to Day 15. On Day 15, TNFα was removed and cells were acutely treated for 3 h with or without FK (1 μM) and/or Bay (10 μM) for TG-hydrolase activity and lipolysis. (A) TG-hydrolase activity. (B) Basal lipolysis. (C) TG-hydrolase activity in response to FK treatment. (D) Lipolytic response to FK treatment. The data are presented as means ± S.E.M. $N = 3–6$. * Significantly different from control condition (C).