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European Journal of Pharmacology 569 (2007) 155–162
www.elsevier.com/locate/ejphar

β-adrenoceptor agonists downregulate adiponectin, but upregulate adiponectin receptor 2 and tumor necrosis factor-α expression in adipocytes

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Received 14 February 2007; received in revised form 26 April 2007; accepted 3 May 2007
Available online 13 May 2007

Abstract

Recently, the insulin-sensitizing adipokine adiponectin and the insulin resistance-inducing adipokine tumor necrosis factor-α (TNF-α) were reported to inhibit each other's production in adipocytes. We investigated the effects of two β_3-adrenoceptor agonists, 5-[(2R)-2-[[(2R)-2-(3-chlorophenyl)-2-hydroxyethyl]amino]propyl]-1,3-benzodioxole-2,2-dicarboxylate (CL-316,243) and (±)-(R*,R*)-[4-[2-[[2-(3-chlorophenyl)-2-hydroxyethyl]amino]propyl]phenoxy]acetic acid (BRL37344), on the gene expression of adiponectin, two adiponectin receptors, and TNF-α in adipose tissues of C57BL/6J mice. CL-316,243 and BRL37344 downregulated adiponectin, but upregulated adiponectin receptor 2 (not receptor 1) in epididymal or/and subcutaneous white adipose tissues and in brown adipose tissue. TNF-α expression was upregulated only in epididymal adipose tissue. To further explore these effects, we treated differentiated 3T3-L1 adipocytes with the non-selective β-adrenoceptor agonist isoproterenol. As a result, adiponectin receptor 2 (but not receptor 1) gene expression and TNF-α protein expression increased, but gene expression and secretion of adiponectin decreased. The upregulation of adiponectin receptor 2 by isoproterenol is most likely via β_2,β_3-adrenoceptors, adenylyl cyclases, and protein kinase A (PKA). However, the accompanying activation of AMP-activated protein kinase (AMPK) may inhibit this upregulation. Our results suggest that upregulation of TNF-α and downregulation of adiponectin by β-adrenoceptor activation may contribute to the pathogenesis of catecholamine-induced insulin resistance, and that upregulation of adiponectin receptor 2 may be a feedback result of reduced adiponectin.

Keywords: β-adrenoceptor; Adiponectin; Adiponectin receptor; Tumor necrosis factor-α; 3T3-L1 adipocyte; Adipose tissue; Catecholamine; Insulin resistance

1. Introduction

White adipose tissue is a key regulator of whole-body energy metabolism, playing a central role in the balance between energy storage and energy mobilization. Brown adipose tissue is specific for metabolic heat production. In rodent adipose tissues, β-adrenoceptors, predominantly β_3-adrenoceptors, are expressed. They mediate the major effects of adrenaline and noradrenaline, such as stimulating lipolysis in white adipose tissue and thermogenesis in brown adipose tissue. Regional differences exist between visceral and subcutaneous white adipose tissues. Visceral fat tissue is known to be lipolytically active in response to catecholamines compared to subcutaneous tissue (Lafontan and Berlan, 2003).

In recent years, it has been demonstrated that adipocytes also secrete biologically active molecules called adipokines. Among them, adiponectin and tumor necrosis factor-α (TNF-α) are two important adipokines that antagonistically influence insulin sensitivity. TNF-α is thought to be a causative factor of insulin resistance in fat tissue, involving insulin receptor autophosphorylation and insulin receptor substrate-1 (IRS-1) tyrosine phosphorylation (Hotamisligil and Spiegelman, 1994; Hotamisligil et al., 1994, 1996). Adiponectin is a recently discovered insulin-sensitizing adipokine composed of an N-terminal collagenous domain and a C-terminal globular domain. In plasma it can exist as full-length adiponectin or globular domain adiponectin (Kadowaki and Yamauchi, 2005; Kadowaki et al., 2006; Fruebis et al., 2001; Yamauchi et al., 2001). Adiponectin stimulates fatty acid oxidation and glucose transport in muscle and inhibit gluconeogenesis in liver

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doi:10.1016/j.ejphar.2007.05.005

by activating AMP-activated protein kinase (AMPK) (Yamauchi et al., 2002). TNF-α and adiponectin are reported to mutually inhibit each other's production in adipose tissue (Maeda et al., 2001, 2002; Fasshauer et al., 2002).

Adiponectin receptor 1 and 2 mediate most effects of adiponectin. Adiponectin receptor 1, which is a high-affinity receptor for globular domain adiponectin and a very low-affinity receptor for full-length adiponectin, is most abundantly expressed in skeletal muscle. Adiponectin receptor 2, which serves as a moderate-affinity receptor for both forms of adiponectin, predominates in liver (Yamauchi et al., 2003). Interestingly, both of these receptors are also expressed in adipocytes, where adiponectin itself is expressed and secreted (Fasshauer et al., 2004). A recent report suggests that adiponectin acts as an autocrine factor in adipocytes (Fu et al., 2005).

Studies demonstrate that β-adrenoceptor agonists decrease adiponectin gene expression via activation of a Gs-protein-PKA-dependent pathway (Fasshauer et al., 2001) in 3T3-L1 adipocytes and in white adipose tissue (Delporte et al., 2002). At the present time, however, little is known about the effects of β-adrenoceptor agonists on the regulation of adiponectin receptors and TNF-α in adipose tissues. Only a single study has reported that chronic treatment of diabetic (db/db) mice with β_3-adrenoceptor agonist increased adiponectin levels and decreased insulin levels in plasma, but left adiponectin receptors in adipose tissues unchanged (Oana et al., 2005). These results may only reflect the indirect effects of β_3-adrenoceptor agonists as altered insulin levels (Tsuchida et al., 2004) could, in turn, regulate adiponectin receptors. The direct influences of β-adrenoceptor agonists on adiponectin receptors are still unknown. In the present study, we examined the acute pharmacological effects of β-adrenoceptor agonists on adiponectin, adiponectin receptors and TNF-α expression in white and brown adipose tissues of lean mice *in vivo* and in differentiated 3T3-L1 adipocytes *in vitro*.

2. Materials and methods

2.1. Materials

Dulbecco's modified Eagle's medium and fetal bovine serum were purchased from Gibco (Rockville, MD). H-89 and compound C were obtained from D. Western Therapeutics Institute, Inc. (Nagoya, Japan) and Calbiochem (La Jolla, CA), respectively. The following chemicals were purchased from Sigma Chemical Co. (St. Louis, MO): 5-[(2R)-2-[[(2R)-2-(3-chlorophenyl)-2-hydroxyethyl]amino]propyl]-1,3-benzodioxole-2,2-dicarboxylate (CL-316,243), (±)-(R*,R*)-[4-[2- [[2-(3-chlorophenyl)-2-hydroxyethyl]amino]propyl]phenoxy]acetic acid (BRL37344), isoproterenol, dobutamine, fenoterol, forskolin, 5-aminoimidazole-4-carboxamide-riboside (AICAR), insulin, isobutylmethylxanthine and dexamethasone.

2.2. Animals and treatments

Twenty-three male C57BL/6J mice aged 10 weeks were obtained from Charles River Labs (Tokyo, Japan) and were fasted for 8 h before the experiments began.

Mice were injected with β_3-adrenoceptor agonist CL-316,243 ($n=8$) or BRL37344 ($n=7$) subcutaneously at a dose of 2 mg/kg of body weight. A second dose was given 8 h later. Control mice ($n=8$) received vehicle (0.9% saline) only.

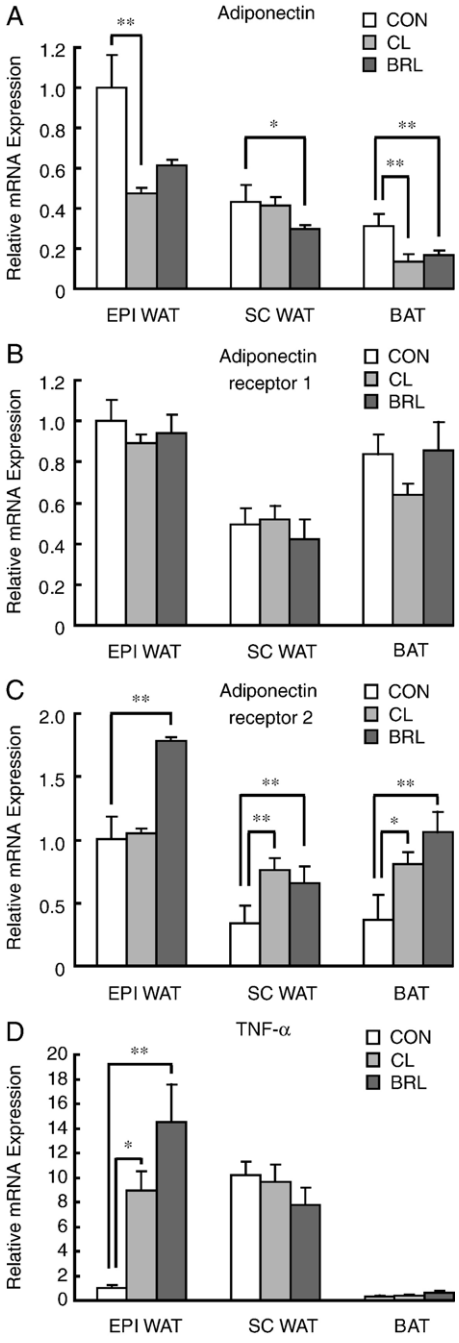

Fig. 1. The effects of two β_3-adrenoceptor agonists, CL-316,243 (CL) and BRL37344 (BRL), on (A) adiponectin, (B) adiponectin receptor 1, (C) adiponectin receptor 2 and (D) TNF-α gene expression in epididymal white adipose tissue (EPI WAT), subcutaneous adipose tissue (SC WAT), and brown adipose tissue (BAT) in C57BL/6J mice. Mice were injected subcutaneously with two doses (2 mg/kg) of CL-316,243, BRL37344 or vehicle. Adipose tissues were collected and the mRNA expression of adiponectin, adiponectin receptor 1, adiponectin receptor 2 and TNF-α were quantified by real-time PCR and normalized to 18S rRNA mRNA levels. Results are expressed as the means ± S.E.M. Open bars, control (CON) mice ($n=8$); light gray bars, CL316,243-treated mice ($n=8$); dark gray bars, BRL37344-treated mice ($n=7$). *$P<0.05$, **$P<0.01$.