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Activation of type 2 cannabinoid receptors (CB2R) promotes fatty acid oxidation through the SIRT1/PGC-1α pathway

Xuqin Zheng [a], Tao Sun [b], Xiaodong Wang [a,*]

[a] Department of Endocrinology, First Affiliated Hospital, Nanjing Medical University, Nanjing, Jiangsu Province 210029, China
[b] Department of Neurology, Jinling Hospital, Nanjing University School of Medicine, Nanjing, Jiangsu Province 210002, China

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ABSTRACT

Abnormal fatty acid oxidation has been associated with obesity and type 2 diabetes. At the transcriptional level, peroxisome proliferator-activated receptor-gamma coactivator 1α (PGC-1α) has been reported to strongly increase the ability of hormone nuclear receptors PPARα and ERRα to drive transcription of fatty acid oxidation enzymes. In this study, we report that a specific agonist of the type 2 cannabinoid receptor (CB2R) can lead to fatty acid oxidation through the PGC-1α pathway. We have found that CB2R is expressed in differentiated C2C12 myotubes, and that use of the specific agonist trans-caryophyllene (TC) stimulates sirtuin 1 (SIRT1) deacetylase activity by increasing the phosphorylation of cAMP response element-binding protein (CREB), thus leading to increased levels of PGC-1α deacetylation. This use of TC treatment increases the expression of genes linked to the fatty acid oxidation pathway in a SIRT1/PGC-1α-dependent mechanism and also drastically accelerates the rate of complete fatty acid oxidation in C2C12 myotubes, neither of which occur when CB2R mRNA is knocked down using siRNA. These results reveal that activation of CB2R by a selective agonist promotes lipid oxidation through a signaling/transcriptional pathway. Our findings imply that pharmacological manipulation of CB2R may provide therapeutic possibilities to treat metabolic diseases associated with lipid dysregulation.

1. Introduction

The increasing occurrence of obesity presents a global health problem which correlates with the growing incidence of conditions such as type 2 diabetes [1]. Fatty acids are central structural and functional components of lipid metabolism and the abnormal oxidation of fatty acids has been associated with obesity and type 2 diabetes [2]. Fatty acid oxidation rates are regulated at both transcriptional and non-transcriptional levels. At the transcriptional level, peroxisome proliferator-activated receptor-gamma coactivator 1α (PGC-1α) was reported to strongly increase the ability of the hormone nuclear receptors PPARα and ERRα to promote transcription of fatty acid oxidation enzymes [3]. The activity of PGC-1α is regulated by deacetylase Sirtin1 (SIRT1) [4]. Previous studies have shown that SIRT1 deacetylase activity is increased through different nutrient and signaling pathways, including glucose restriction, AMP-activated protein kinase (AMPK) activation and cAMP response element-binding protein (CREB) activation [5]. SIRT1/PGC-1α pathway has been considered to be an important mediator of fatty acid oxidation.

During the past decade, the biological effects of cannabinoids have been attracting more and more interest. Two main subtypes of cannabinoid receptors, type 1 cannabinoid receptors (CB1R) and type 2 cannabinoid receptors (CB2R), have been identified in mammalian tissues. Selective agonists and antagonists for these receptors have been identified [6]. CB1R is reported to be highly expressed in the brain [6], while CB2R was initially considered to be primarily expressed in immune cells [7]. However, recent studies have intriguingly demonstrated that this receptor can also be found in the brain [8], in endothelial cells of various origins [9], and in vascular smooth muscle cells [10]. A bicyclic sesquiterpene, trans-caryophyllene (TC), has been reported to be a CB2R selective agonist, which binds to CB2R but not CB1R and results in activation of the Gi/Go subtype of G proteins [11]. A previous study has demonstrated that TC could produce neuroprotective effects in ischemic models both *in vitro* and *in vivo* by activating CB2Rs. Further study has verified that increased levels of AMPK and phosphorylation of CREB are both involved [12]. However, the effects of CB2R agonists on SIRT1 deacetylase, PGC-1α activity, and fatty acid oxidation rates are as yet unknown. In this study, we identify CB2R expression at the mRNA and protein level in myotubes, however we do not see CB1R expression at the protein level. Addition of a known CB2R agonist, TC, stimulates SIRT1 deacetylase activity by increasing the phosphorylation of CREB. As a result, PGC-1α is deacetylated and activated, resulting in an increase in the expression of genes linked to complete oxidation of fatty acids. Confirmation that this agonist works through CB2R was

* Corresponding author. Address: No. 300, GuangZhou Road, Gulou District, Nanjing, Jiangsu Province 210029, China. Fax: +86 025 83718842.
E-mail address: xdwang666@hotmail.com (X. Wang).

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demonstrated by selective knockdown of CB2R which resulted in the loss of TC activity on the activation of SIRT1/PGC-1α and oxidation of fatty acids.

2. Materials and methods

2.1. Cell culture

C2C12 cells of mice were grown in Dulbecco's modified low-glucose Eagle (DMEM) medium containing 10% FBS (v/v), 4.0 mM glutamine, and 1% penicillin and streptomycin. Cells were cultured in a humidified incubator with 5% CO_2 at 37 °C. When cells were 70–80% confluent, the differentiation of myoblasts into myotubes was induced by switching the medium to DMEM containing 2% horse serum (GIBCO, Grand Island, NY, USA) for 72 h. Cells were treated with 1 μM trans-caryophyllene (TC) (Sigma–Aldrich, St. Louis, MO, USA) for 48 h. Cellular transfection to knockdown CB2R protein expression was performed using Lipofectamine RNAiMAX (Invitrogen) according to the manufacturer's instructions. Briefly, 24 h before transfection, cells (5×10^5) were plated in 0.5 ml cultural medium and allowed to grow overnight at 37 °C and 5% CO_2. The small interfering RNA (siRNA) transfection complex, formed by combining transfection reagent and 50 nM siRNA (QIAGEN, USA; Ctrl_Allstars_1 Negative Control (Neg siRNA) or Mm_CNR2_9 target sequence: AAGGCCCAAGGTCCTCGGTTA) in serum-free Opti-MEM Medium, was added dropwise to cells. The successful knockdown of CB2R was verified by western blot analysis.

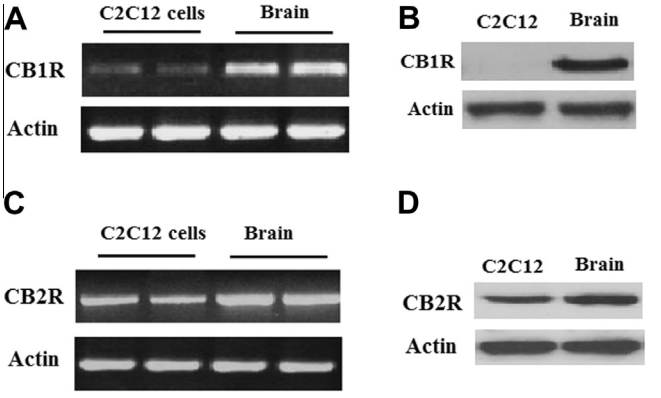

Fig. 1. Differentiated C2C12 myotubes express CB2R. (A) RT-PCR for expression of CB1R, with brain samples used as a positive control and actin serving as the housekeeping gene. (B) CB1R was not observed by western blot analysis. (C) RT-PCR for expression of CB2R, with brain samples used as a positive control and actin serving as the housekeeping gene. (D) Western blot analysis revealed that CB2R was expressed in C2C12 myotubes.

2.2. Reverse transcriptase polymerase chain reaction (RT-PCR)

Total RNA from cultured cells was isolated using Trizol reagent (Invitrogen) in accordance with the manual instructions. Two micrograms of total RNA was used to synthesize cDNA using a high capacity cDNA reverse transcription kit (Applied Biosystems, Foster, CA, USA). Synthesized cDNA by reverse transcriptase polymerase chain reaction (RT-PCR) was used to study the expression of CB1R and CB2R in C2C12 cells. The primers used for CB1R are: forward (5′-CCAAGAAAAGATGACGGCAG-3′) and reverse (5′-AGGATGACACATAGCACCAG-3′). The primers used for CB2R are: forward (5′-TCGCTTACATCCTTCAGACAG-3′) and reverse (5′-TCTT CCCTCCCAACTCCTTC-3′). The primers used for β-actin are: forward (5′-CTGTCGAGTCGCGTCCACCC-3′), and reverse (5′-GCTTTGCACA TGCCGGAGCC-3′).

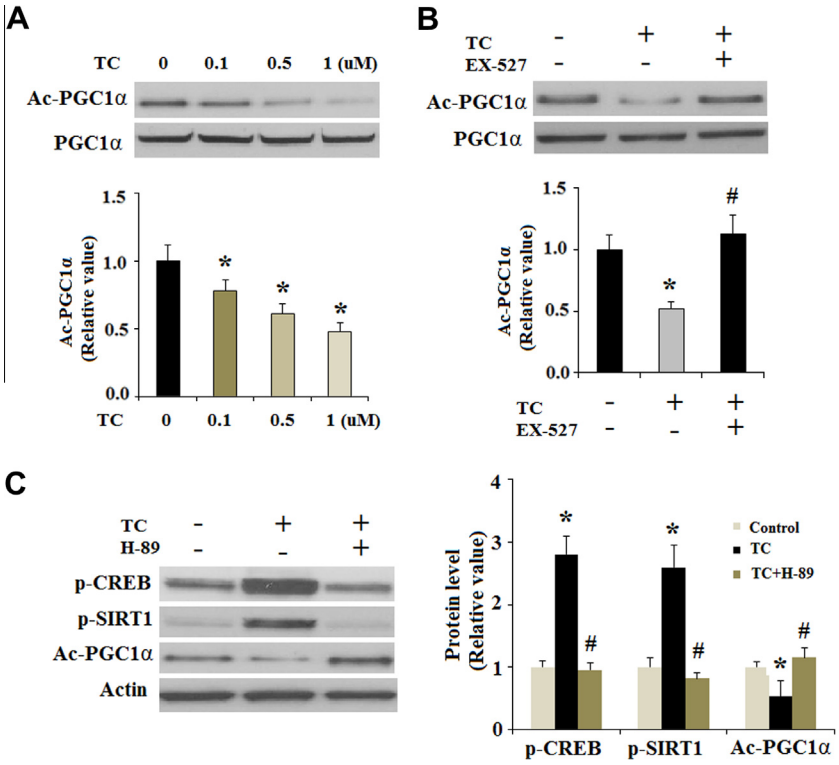

Fig. 2. TC, as a specific agonist of CB2R, induces PGC1α deacetylation through SIRT1 in C2C12 myotubes. (A) TC deacetylates PGC-1α in a concentration-dependent manner. Acetylated PGC-1α was detected using the acetyl-lysine-specific antibody following PGC-1α immunoprecipation ($^*p < 0.01$ vs. non-treatment group); (B) TC deacetylates PGC-1α in a SIRT1-dependent manner. C2C12 myotubes were treated with 0.5 μM TC, with control cells pretreated with EX-527 (2 μM) for 4 h before the addition of TC ($^*p < 0.01$ vs. non-treatment group; $^\#p < 0.01$ vs. TC treatment group); (C) TC induces phosphorylation of the PKA substrate p-CREB. The effects of TC on phosphorylation of SIRT1 and deacetylation of PGC-1α were abolished by the PKA inhibitor H-89 ($^*p < 0.01$ vs. control group; $^\#p < 0.01$ vs. TC treatment group).