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ScienceDirect
Regulatory Peptides 139 (2007) 72–79
REGULATORY PEPTIDES
www.elsevier.com/locate/regpep

Adiponectin modulates carnitine palmitoyltransferase-1 through AMPK signaling cascade in rat cardiomyocytes

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Received 12 March 2006; received in revised form 3 October 2006; accepted 3 October 2006
Available online 15 November 2006

Abstract

Adiponectin, an adipocyte-derived polypeptide hormone, plays an important role in regulating fatty acid oxidation. β-oxidation of fatty acids supplies most of the cardiac energy and carnitine palmitoyltransferase (CPT)-1 serves as a key regulator during this process. To characterize the potential effects of adiponectin on CPT-1, we incubated rat neonatal cardiomyocytes with globular adiponectin (gAd). Results showed that gAd promoted the activity and mRNA expression of CPT-1. The underlying signal pathway involved in this modulatory effect was further investigated. Inhibition of AMP-activated protein kinase (AMPK) with adenine 9-β-D-arabinofuranoside (AraA) completely abrogated gAd-mediated AMPK and acetyl coenzyme A carboxylase (ACC) phosphorylation and suppressed the promotion of CPT-1 activity. gAd also induced the phosphorylation of p38 mitogen-activated protein kinase (MAPK) and peroxisome proliferator-activated receptor (PPAR)-α, which was inhibited by AraA. SB202190, a p38MAPK inhibitor, blocked gAd-stimulated PPAR-α phosphorylation. When AMPK and/or p38MAPK was inhibited, gAd-enhanced mRNA expression of CPT-1 was partially reduced. In conclusion, our study suggests that the activation of AMPK signaling cascade participates in the promotion effect of gAd on CPT-1.

Keywords: Globular adiponectin (gAd); Carnitine palmitoyltransferase-1 (CPT-1); AMP-activated protein kinase (AMPK); p38 mitogen-activated protein kinase (p38MAPK); Peroxisome proliferator-activated receptor-α (PPAR-α); Cardiomyocytes

1. Introduction

Adiponectin, an adipocyte-derived hormone, is present abundantly in circulating blood [1,2]. It is defined as a 244-amino acid polypeptide with a collagen-like domain and a C1q-like globular domain. The globular domain, also named globular adiponectin (gAd), is the biological active domain [3]. Recently, adiponectin has been identified as one of the adipocytokines with important metabolic effects [4–9]. Injection of recombinant gAd enhances fatty acid oxidation and ameliorates insulin resistance in *ob*/*ob* mice [10]. Furthermore, fatty acid oxidation is promoted when rat skeletal muscle is incubated with gAd [11]. In agreement with these findings, cellular treatment with either gAd or full-length adiponectin stimulates fatty acid oxidation in C2C12 myocytes [12]. It is well established that the activation of AMP-activated protein kinase (AMPK) participates in the promotion effect of gAd on fatty acid oxidation in liver and skeletal muscle [11,13].

Mitochondrial β-oxidation of long-chain fatty acids is the major source of cardiac energy. Prior to undergoing β-oxidation in mitochondrial matrix, the long-chain fatty acyl-CoA must be transferred from cytosol into matrix. Carnitine palmitoyltransferase (CPT)-1, located on the outer mitochondrial membrane, catalyzes the formation of long-chain fatty acyl–carnitine from long-chain fatty acyl-CoA and carnitine. The acyl–carnitine complexes are then transported across the inner mitochondrial membrane. Therefore, CPT-1 serves as a key regulator in controlling the rate of fatty acid uptake by mitochondria [14,15]. It has been demonstrated that AMPK causes phosphorylation and inhibition of acetyl coenzyme A carboxylase (ACC), which reduces the production of malonyl-CoA, an allosteric inhibitor of CPT-1 [16]. Thus AMPK activation appears to be extremely important in regulating CPT-1 activity.

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0167-0115/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.regpep.2006.10.007

It has been shown that gAd increases fatty acid oxidation in isolated perfused newborn rabbit heart, suggesting the close relationship between gAd and fatty acid metabolism in heart [17]. However, little is known about the effect of gAd on CPT-1, a key enzyme in controlling cardiac fatty acid oxidation. Moreover, Shibata et al. have revealed that full-length adiponectin activates AMPK in neonatal rat cardiomyocytes [18]. This prompts us to assume that AMPK may be a connection between gAd and CPT-1. The present work was to study the modulatory effect of gAd on CPT-1 and the underlying signal pathway in neonatal rat cardiomyocytes.

2. Materials and methods

2.1. Reagents

Human recombinant gAd was purchased from Phoenix Pharmaceuticals Inc. (San Francisco, CA). Anti-phospho-AMPK and anti-phospho-ACC antibodies were from Cell Signaling Technology (Beverly, MA). Anti-phospho-p38MAPK, anti-p38MAPK, anti-AMPK, anti-ACC and anti-PPAR-α antibodies were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). Anti-phospho-PPAR-α antibody was from Affinity BioReagents (Golden, CO). Adenine 9-β-D-arabinofuranoside (AraA), palmitoyl-CoA and SB202190 were purchased from Sigma-Aldrich (St. Louis, MO). 5-Aminoimidazole-4-carboxamide-1-4-ribofuranoside (AICAR) was obtained from Toronto Research Chemicals (Toronto, Canada).

2.2. Primary culture of neonatal rat ventricular myocytes

Neonatal rat ventricular myocytes (NRVMs) were prepared according to the method described by Komuro et al. [19]. Ventricles of 1–3 day-old Wistar rats were minced and digested in phosphate-buffered saline (PBS) containing 0.1% trypsin (Gibco BRL) and 0.05% type I collagenase (Gibco BRL) at 37 °C. The cells were pelleted by low speed centrifugation and suspended in Dulbecco's modified Eagle's medium (DMEM, Gibco BRL) containing 15% fetal bovine serum (FBS, Gibco BRL). A single preplating step was used to further increase the ratio of cardiomyocytes to noncardiomyocytes. Noncardiomyocytes attached readily to the bottom of culture dishes. The remaining unattached myocytes were then seeded at 1×10^5 cells per cm^2 in 60 mm dishes and were kept in 5% CO_2 at 37 °C. The identity of NRVMs was confirmed by morphological examination and by staining with anti-sarcomeric α-actin antibody. Most (>95%) of the cells were identified as cardiomyocytes. Experiments were performed 3–4 days after cell plating. Cells were placed in a serum-free medium for 24 h prior to experiments.

2.3. Extraction of cardiac mitochondria and assay of CPT-1 activity

Mitochondria in NRVMs were isolated according to Yao et al. [20]. NRVMs were homogenized in a medium containing 300 mM sucrose, 5 mM MOPS, 1 mM EGTA, 5 mM K_2HPO_4, and 0.1% BSA, pH 7.4 at 4 °C. The homogenate was centrifuged at 1500 ×*g* for 10 min (4 °C). The supernatant was collected and centrifuged at 9800 ×*g* for 5 min (4 °C). The mitochondrial pellet was washed and centrifuged twice in the same medium. The protein concentration was determined by Lowry's method [21]. Mitochondrial protein (1 mg/ml) was incubated in 1 ml of a medium containing 150 mM sucrose, 60 mM KCl, 25 mM Tris/HCl, 1 mM EDTA, 0.1 mM 4, 4′ dithiodipyridine, and 1.3 mg/ml BSA at 37 °C for 2 min. The enzymatic reaction was initiated by adding palmitoyl-CoA (100 μM) and carnitine (400 μM) to generate palmitoylcarnitine, and the mixture was incubated at 37 °C for 3 min. CPT-1 activity, determined by the initial rate of decrease in palmitoyl-CoA, was assessed using spectrophotometry at 324 nm according to Mohamed et al. [22].

2.4. Reverse-transcription polymerase chain reaction (RT-PCR)

Total RNA of NRVMs was isolated using Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. cDNA was then generated from total RNA with M-MLV reverse transcriptase (Promega, Madison, WI). PCR was carried out with an initial denaturing at 94 °C for 1 min, followed by 30 cycles consisting of denaturing at 94 °C for

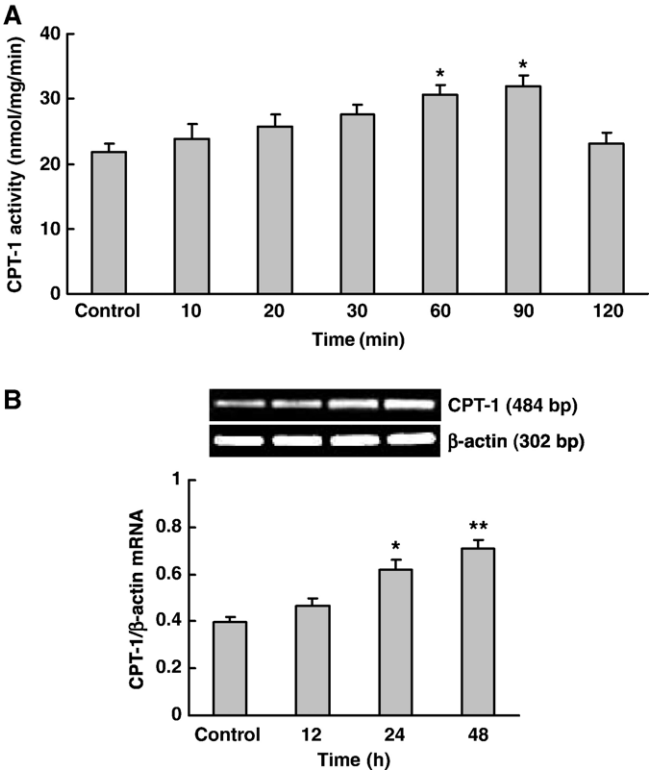

Fig. 1. gAd promoted the activity and mRNA expression of CPT-1. (A) NRVMs were incubated with 1 μg/ml gAd for 10–120 min. Cardiac mitochondria were extracted and CPT-1 activities were assessed using spectrophotometry at 324 nm. (B) NRVMs were treated with 1 μg/ml gAd for 12–48 h, after which the total RNA was used for RT-PCR analysis with CPT-1 and β-actin primer pairs. The expression levels of CPT-1 were quantified by scanning densitometry and normalized by β-actin. Data are means±SE of three independent experiments. *$P<0.05$, **$P<0.01$ vs. control.