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Regulation of adiponectin receptor 1 in human hepatocytes by agonists of nuclear receptors

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Abstract

The adiponectin receptors AdipoR1 and AdipoR2 have been identified to mediate the insulin-sensitizing effects of adiponectin. Although AdipoR2 was suggested to be the main receptor for this adipokine in hepatocytes, AdipoR1 protein is highly abundant in primary human hepatocytes and hepatocytic cell lines. Nuclear receptors are main regulators of lipid metabolism and activation of peroxisome proliferator-activated receptor α and γ, retinoid X receptor (RXR), and liver X receptor (LXR) by specific ligands may influence AdipoR1 abundance. AdipoR1 protein is neither altered by RXR or LXR agonists nor by pioglitazone. In contrast, fenofibric acid reduces AdipoR1 whereas hepatotoxic troglitazone upregulates AdipoR1 protein in HepG2 cells. Taken together this work shows for the first time that AdipoR1 protein is expressed in human hepatocytes but that it is not a direct target gene of nuclear receptors. Elevated AdipoR1 induced by hepatotoxic troglitazone may indicate a role of this receptor in adiponectin-mediated beneficial effects in liver damage.

Keywords: Adiponectin; 9-*cis*-Retinoic acid; Hepatocyte; 25-Hydroxycholesterol; Retinoid X receptor; Liver X receptor; Peroxisome proliferator-activated receptor γ; AdipoR1

Adiponectin is highly abundant in human serum and is secreted by adipose tissue in inverse proportion to the body mass index [1]. Adiponectin improves whole body insulin sensitivity and in addition exerts anti-inflammatory effects by reducing NF-kB activation [2]. Recent studies indicate that adiponectin can prevent fatty liver disease. In rat hepatocytes, adiponectin stimulates mitochondrial β-oxidation and downregulates sterol regulatory element-binding protein-1c (SREBP-1c) levels thereby reducing triglyceride storage in the liver [3]. This finding is supported by studies in which recombinant adiponectin given to ob/ob mice ameliorated hepatic steatosis and normalized alanine aminotransferase (ALT) levels [4]. Besides these protective effects on fatty liver disease, adiponectin attenuates T-cell mediated hepatic inflammation by reducing the release of proinflammatory cytokines, the activation of hepatic stellate cells, and cell death of hepatocytes [5].

Recently, two 7-transmembrane proteins, AdipoR1 and AdipoR2, have been identified to function as adiponectin receptors [6]. AdipoR1 mRNA is mainly expressed in the human heart and skeletal muscle, whereas AdipoR2 mRNA is predominantly found in the liver [6]. Trimeric, hexameric, and higher molecular weight isoforms of adiponectin have been described.

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Trimeric and oligomeric adiponectin act mainly on the liver, and activation of the AMP-activated protein kinase (AMPK) and PPARα has been demonstrated [6].

AdipoR1 and AdipoR2 mRNA expression was investigated in hepatocytes and insulin was found to suppress mRNA expression of these receptors [7]. In fasted mice, the mRNAs were found induced in the liver, and refeeding of these animals decreased AdipoR1 and AdipoR2 expressions. Insulin deficiency led to an upregulation of the receptors, whereas insulin replenishment in these mice reduced AdipoR1 and AdipoR2 mRNA [7]. These data indicate that a reduced expression of adiponectin receptors may contribute to fatty liver disease in obesity and the metabolic syndrome. Steatotic liver is more susceptible to liver fibrosis associated with activation of hepatic stellate cells (HSC) and hepatocytes death [8]. AdipoR1 and AdipoR2 proteins were detected in hepatic stellate cells and activation led to a decreased abundance of AdipoR1 whereas AdipoR2 was not altered [9]. Therefore not only reduced circulating adiponectin but also downregulation of the corresponding receptors may contribute to fatty liver disease or even liver fibrosis.

So far the expression of AdipoR1 protein in human hepatocytes was not investigated. Therefore, we determined AdipoR1 mRNA and protein in hepatocytic cell lines and primary hepatocytes. The influence of agonists of the nuclear receptors PPARα and PPARγ, RXR and LXR on the expression of AdipoR1 was studied.

Materials and methods

Culture media and reagents. Dulbecco's modified Eagle's medium (DMEM, high glucose) was from PAA (Karlsruhe, Germany), RNeasy Mini Kit was from Qiagen (Hilden, Germany), and oligonucleotides were synthesized by Metabion (Planegg-Martinsried, Germany). LightCycler FastStart DNA Master SYBR Green I was purchased from Roche (Mannheim, Germany). Troglitazone, 9-*cis*-retinoic acid (9-cRA), 25-hydroxycholesterol (25-OH), fenofibric acid, and anti-β-actin antibody were ordered from Sigma (Deisenhofen, Germany), and Pioglitazone was kindly provided by Takeda Pharma GmbH (Aachen, Germany). Recombinant adiponectin was produced in High Five insect cells. AdipoR1 peptide antibody was raised in a rabbit and affinity purified (Pineda Antibody Services, Berlin, Germany). Anti-V5 antibody was from Invitrogen (Karlsruhe, Germany), ABCA1 antibody was from Abcam (Cambridge, USA), and the TNT Quick Coupled Transcription/Translation system was ordered from Promega (Mannheim, Germany).

Primary cells. Primary hepatocytes were isolated and cultivated as described before [10]. Tissue samples from human liver resection were obtained from patients undergoing partial hepatectomy. Experimental procedures were performed according to the guidelines of the charitable state controlled foundation HTCR (human tissue and cell research), with the informed patient's consent [11] approved by the local ethical committee of the University of Regensburg.

Monitoring of gene expression by real time RT-PCR. Real time PCR was performed as recently described [12]. The primers for AdipoR1 were: AdipoR1uni: 5′-GGG GAA TTC TCT TCC CAC AAA GGA TCT GTG GTG-3′ and AdipoR1 rev: 5′-GGG CTG CAG TTA AGT TTC TGT ATG AAT GCG GAA GAT-3′. For β-actin: β-actin uni 5′-CTA CGT CGC CCT GGA CTT CGA GC-3′ and β-actin rev: 5′-GAT-GGA GCC GCC GAT CCA CAC GG-3′ were used.

Amplification in the LightCycler capillaries was for 45 cycles for AdipoR1 and 40 cycles for β-actin with initial incubation of 10 min at 95 °C for activation of *Taq* polymerase. Cycling parameters were 15 s at 95 °C, 10 s at 60 °C, and 10 s at 72 °C. Fluorescence was monitored at 83 °C for AdipoR1 and 86 °C for β-actin. The second derivative method was used for quantification with the LightCycler software. For quantification of the results, the standard curve method was used. Normalization was performed by dividing each value calculated for AdipoR1 by the value of the corresponding housekeeping gene.

SDS–PAGE and immunoblotting. The cells were harvested, washed in PBS, and solubilized in RIPA buffer. Proteins were separated by SDS–polyacrylamide gel electrophoresis and were transferred to PVDF membranes (Bio-Rad, Germany). Incubations with antibodies were performed in 1% BSA in PBS, 0.1% Tween. Detection of the immune complexes was carried out with the ECL Western blot detection system (Amersham Pharmacia, Deisenhofen, Germany).

Statistical analysis. Data are represented as Box Plots indicating the median, the upper and lower quartile, and the largest and the lowest value in the data set. Statistical differences were analyzed by Student's t test and a value of $P < 0.05$ was regarded as statistically significant.

Results

Expression of AdipoR1 in human hepatocytic cell lines and primary hepatocytes

AdipoR1 mRNA expression was analyzed in the human hepatoma cell lines HepG2 and PLC/PRF/5, and primary hepatocytes from five different donors. AdipoR1 is equally abundant in HepG2 and PLC/PRF/5 cells, and is slightly but significantly lower in primary cells (Fig. 1A). To investigate AdipoR1 protein, an AdipoR1 specific antibody was generated. In vitro translated AdipoR1 and AdipoR2 proteins with a C-terminal V5 tag was used for immunoblot using an anti-V5 antibody or anti-AdipoR1 antibody (Fig. 1B). Anti-AdipoR1 antibody specifically detects AdipoR1 protein and therefore was used for immunoblots. Twenty microgram protein isolated from primary hepatocytes of three different donors and 10 μg protein from HepG2 or PLC/PRF/5 were used. AdipoR1 protein was detected in all samples but protein levels are lower in primary hepatocytes (Fig. 1C).

Expression of AdipoR1 in human tissues and different cell lines

Expression of AdipoR1 protein was investigated in several cell lines using immunoblot. AdipoR1 was found expressed in the epithelioid carcinoma cell line HeLa and the colon carcinoma cell line Caco-2. AdipoR1 was very lowly abundant in the chinese hamster ovary cell line CHO (Fig. 2A). Immunoblot using human tis-