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別紙１

厚生労働科学研究研究費補助金

ヒトゲノム・再生医療等研究事業

生活習慣病の鍵分子、アディポネクチン受容体の病態生理的意義と情報伝達経路の解明

平成１７年度　総括研究報告書

主任研究者　山内　敏正

平成１８（２００６）年　４月

別紙２

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別紙3

厚生労働科学研究費補助金（ヒトゲノム・再生医療等研究事業）
（総括・分担）研究報告書

生活習慣病の鍵分子、アディポネクチン受容体の病態生理的意義と情報伝達経路の解明
（分担研究分：アディポネクチン受容体欠損マウス解析）

（主任）研究者　山内　敏正　東京大学医学部附属病院
統合的分子代謝疾患科学講座　客員助教授

研究要旨：(1)組織特異的過剰発現と一部前倒しとなる遺伝子欠損による機能解析により、AdipoR1はアディポネクチンによるAMP キナーゼ活性化の情報伝達経路と、AdipoR2 は 肝臓においてPPARα活性化の経路と、それぞれより強くリンクしている可能性を明らかにした。 (2)アディポネクチン受容体の細胞内情報伝達の鍵となる結合蛋白として、チロシンリン酸化蛋白との結合部位（Phosphotyrosine binding domain）、リン酸化脂質との結合部位（Pleckstrin domain）、Aktとの結合部位、さらにLeucine zipper domainを持つPPALなどを同定した。

分担研究者氏名・所属機関名及び所属機関における職名：
所属機関：東大病院
窪田直人(客員助手)、植木浩二郎(特任助教授)、門脇 孝(教授)

A．研究目的
(1)遺伝子改変マウスの解析によりアディポネクチン受容体の生理的・病態生理的意義を解明する。
(2)アディポネクチン受容体結合蛋白の同定とそれに基づいた細胞内情報伝達機構の解明

B．研究方法
相同組換えを用いた定法によって、AdipoRの欠損マウスを作製した。野生型マウスおよびAdipoR1欠損マウスにおいて、糖負荷試験を施行した。AMPKのリン酸化をウエスタンブロッティング法にて検討した。脂肪の合成や燃焼および糖新生に関わる遺伝子の発現はTaqMan PCR法にて検討した。

（倫理面への配慮）
実験動物については「実験動物の飼養及び保管等に関する基準」（昭和55年3月27日 総理府告示）を遵守して実験に利用し、実験に伴う苦痛を最小限にするために適当な処置を行うこととする。本学に設置された動物実験施設の承認を得て許可された研究計画書に従い研究を実施する。動物実験の取り扱いに関しては倫理指針を遵守する。

E．結論
(1) AdipoR1はアディポネクチンによるAMP キナーゼ活性化の情報伝達経路と、AdipoR2 は 肝臓においてPPARα活性化の経路と、それぞれより強くリンクしている可能性を明らかにした。
(2)アディポネクチン受容体の細胞内情報伝達の鍵となる結合蛋白としてPPALなどを同定した。

F．健康危険情報
なし。

G．研究発表
1．論文発表
Tsuchida A, et al. Peroxisome proliferator-activated receptor (PPAR){alpha} activation increases adiponectin receptors and reduces obesity- related inflammation in adipose tissue: Comparison of activation of PPAR {alpha},PPAR{gamma}, and their combination. Diabetes 54(12);3358-3370, 2005.

Kubota N, et al. Pioglitazone ameliorates insulin resistance and independent pathway. J Biol Chem. 281;8748-8755, 2006.

Kadowaki T, et al. Adiponectin and

C．研究結果
全体総括：(1)組織特異的過剰発現と一部前倒しとなる遺伝子欠損による機能解析により、AdipoR1はアディポネクチンによるAMP キナーゼ活性化の情報伝達経路と、AdipoR2 は 肝臓においてPPARα活性化の経路と、それぞれより強くリンクしている可能性を明らかにした。
(2)アディポネクチン受容体の細胞内情報伝達の鍵となる結合蛋白としてPPALなどを同定した。

分担研究部分：アディポネクチン受容体欠損マウス解析・解明を試みた。組織特異的過剰発現と一部前倒しとなる遺伝子欠損による機能解析により、AdipoR1はアディポネクチンによるAMP キナーゼ活性化の情報伝達経路と、 AdipoR2 は肝臓においてPPARα活性化の経路と、それぞれより強くリンクし、インスリン抵抗性を改善させている可能性を明らかにした。さらに、生体内でAdipoR1はアディポネクチンによるAMPK活性化を介して、糖新生の抑制、脂肪合成の抑制、脂肪酸燃焼の促進を担っていることを示した。一方AdipoR2はPPARαを介して、脂肪酸燃焼とエネルギー消費の促進を担っていることを示した。また、生体内でAdipoR1・R2はアディポネクチンの特異的結合に、AdipoR1はアディポネクチンによるAMPK活性化に、必須であることを示した。

D．考察
AdipoR1・AdipoR2を過剰発現させることが出来るアデノウイルス、及びAdipoR1・AdipoR2のノックアウトマウスは、AdipoR1・AdipoR2の生理機能・病態生理的意義を明らかにすることが出来、新たな抗生活習慣病の原因の分子メカニズムの解明、及びそれに基づいた新規の治療法の可能性を提供することが出来るものと考えられる。また、それら抗生活習慣病治療における化合物の生体内における評価に活用することが出来るため、非常に価値が高いものと考えられるので、特許の申請を行ったうえで、最大限活用・提供していく。また、AdipoR1に結合するPPALがAMPK活性化に重要であるという研究成果は、PPAL を分子標的とした抗生活習慣病治療開発の可能性を提供するので、活用していく。

adiponectin Receptors.
Endocr Rev. 26(3); 439-451, 2005.

2. 学会発表
「アディポネクチン受容体AdipoR１・R2の生理的･病態生理学的意義の解明」
第102回日本内科学会
大阪国際会議場（グランドキューブ大阪）
平成１７年４月７日（ポスター発表）

「アディポネクチン・アディポネクチン受容体の病態生理的意義」
第48回日本糖尿病学会
ポートピアホテル
平成１７年５月１４日

「脂肪細胞由来の抗糖尿病・抗動脈硬化ホルモン,アディポネクチンの受動態同定と作用メカニズム･病態生理学的意義の解明」
第26回日本肥満学会
ホテルロイトン札幌
平成１７年１０月１３日

「Adiponectin and AdipoRs: new frontier in a strategy for treatment of metabolic syndrome」
第26回日本肥満学会
ホテルロイトン札幌
平成１７年１０月１３日

「アディポネクチン」
第35回日本心脈管作動物質学会
栃木県総合文化センター
平成１８年２月１８日

「Osmotin, that is a Ligand for the Yeast Homolog of AdipoR, Activates AMP kinase Via AdipoR in Myocytes」
Purdue University 講演会
Purdue University
平成１７年６月９日

「Pathophysiological Roles of Adiponectin Receptors AdipoRs」
第65回アメリカ糖尿病学会
Convention Center
平成１７年６月１０日～６月１３日

「Pathophysiological roles of adiponectin receptors AdipoRs」
Keystone Symposia
Vancouver BC, Canada
平成１８年１月２２日

別紙3

厚生労働科学研究費補助金（ヒトゲノム・再生医療等研究事業）
（分担）研究報告書

アディポネクチン受容体組織特異的過剰発現マウス解析

（分担）研究者　窪田　直人　東京大学医学部附属病院
統合的分子代謝疾患科学講座　客員助手

研究要旨：【目的】糖尿病モデルマウスの肝臓にアディポネクチン受容体（AdR）を過剰発現させ、その表現型を解析した。【方法】アデノウイルスを用いてAdR1,2の発現を増加させ，糖・脂質代謝，AdによるAMPKのリン酸化および各種遺伝子発現に対する影響を検討した。【結果】AdR1の肝臓での過剰発現により、AdによるAMPKの活性化が増強し，糖新生・脂肪合成に関わる分子の発現が低下した。AdR2により、PPARαの標的遺伝子である脂肪燃焼・エネルギー消費に関わる分子の発現が増加した。AdR1またはR2により，脂肪燃焼が促進され、肝臓内中性脂肪含量が低下し、糖尿病モデルマウスの耐糖能障害が改善した。これらAdR過剰発現による効果は、Adの欠損により、消失する傾向が認められた。【考察】AdR1はAdによるAMPK活性化の情報伝達経路と，AdR2は肝臓でのPPARα活性化の経路とそれぞれより強くリンクしている可能性が示唆され，その発現制御が糖尿病の治療法となりうる可能性が考えられた。

A．研究目的

我々は，肥満でアディポネクチンの血中レベルが低下し糖尿病・動脈硬化が惹起される事，およびアディポネクチンの補充がAMPKの活性化などを介してこれら病態の治療法となる事を報告した。肥満においてはAdipoRの発現低下が認められ、アディポネクチン抵抗性が存在することを報告した。本研究ではAdipoRの発現を回復させることが肥満に伴う耐糖能障害を改善させうるかどうか、生体内での病態生理的意義を明らかにするために，肝臓にAdipoRの発現を増加させ、その表現型を解析した。

B．研究方法

アデノウィルスを用いて肥満・２型糖尿病モデルマウスであるdb/dbマウスの肝臓でAdipoRの発現を増加させ、糖・脂質代謝、アディポネクチンによるAMPKの活性化、脂肪の合成や燃焼および糖糖新生に関わる遺伝子の発現レベルや活性を検討した。

E．結論

AdR1はAdによるAMPK活性化の情報伝達経路と，AdR2は肝臓でのPPARα活性化の経路とそれぞれより強くリンクしている可能性が示唆され、その発現制御が糖尿病の治療法となりうる可能性が考えられた。

F．健康危険情報

なし。

G．研究発表

1.　論文発表

Tsuchida A, et al. Peroxisome proliferator-activated receptor (PPAR){alpha} activation increases adiponectin receptors and reduces obesity- related inflammation in adipose tissue:
Comparison of activation of PPAR {alpha},PPAR{gamma}, and their combination.
Diabetes　54(12);3358-3370, 2005.

Kubota N, et al. Pioglitazone ameliorates　insulin resistance and independent pathway.
J Biol Chem. 281;8748-8755, 2006.

（倫理面への配慮）
実験動物については「実験動物の飼養及び保管等に関する基準」（昭和55年3月27日 総理府告示）を遵守して実験に利用し、実験に伴う苦痛を最小限にするために適当な処置を行うこととする。本学に設置された動物実験施設の承認を得て許可された研究計画書に従い研究を実施する。動物実験の取り扱いに関しては倫理指針を遵守する。

C．研究結果
AdipoR1の肝臓への過剰発現は，db/dbマウスの耐糖能障害を改善させた。このときアディポネクチンによる肝臓でのAMPキナーゼの活性化が増強されるのが認められた。AMPキナーゼは肝臓において、糖新生に関わるPEPCKやG6Pase、脂肪合成に関わるSREBP1cのmRNAレベルを低下させる作用や、脂肪酸燃焼を促進することが報告されている。実際、AdipoR1の過剰発現により、PEPCKやG6Pase、SREBP1cのmRNAレベルが低下していること、および肝臓での脂肪酸燃焼の活性が有意に増加し、肝臓内中性脂肪含量が低下する傾向が認められた。一方，AdipoR2の肝臓での過剰発現もdb/dbマウスの耐糖能障害を改善させた。この時、PPARαの標的遺伝子で脂肪酸燃焼に関わるACOやエネルギー消費に関わるUCP2の発現が増加しており、肝臓での脂肪酸燃焼の活性が有意に増加し、肝臓内中性脂肪含量が低下する傾向が認められた。これらAdipoRの効果がアディポネクチンを必要とするかどうか検討する目的に、アディポネクチンを欠損したdb/dbマウスあるいは脂肪組織が消失してアディポネクチンが枯渇している脂肪萎縮糖尿病のモデルであるA-ZIPトランスジェニックマウスでその効果を検討したが、顕著にその効果が消失する傾向が認められた。

D．考察
アデノウイルスによるAdipoR1あるいはAdipoR2の肝臓への過剰発現は肥満に伴う糖尿病を改善させ，その発現制御が糖尿病の治療法となりうる可能性が考えられた。AdipoR1はアディポネクチンによるAMPK活性化の情報伝達経路と，AdipoR2は肝臓でのPPARα活性化の経路と，それぞれより強くリンクしている可能性が示唆された。

2.　学会発表
「アディポネクチン欠損マウスはレプチン感受性を呈する」
（予定を含む。）
第48回日本糖尿病学会
ポートピアホテル
平成１７年５月１４日

「チアゾリジン誘導体の抗糖尿病作用におけるアディポネクチンの役割の解明
第9回日本病態栄養学会年次学会集会
和歌山県民文化会館
平成１８年１月６日

H．知的財産権の出願・登録状況
（予定を含む。）
AdipoR1・AdipoR2を過剰発現させることが出来るアデノウィルスの特許準備中

厚生労働科学研究費補助金（ヒトゲノム・再生医療等研究事業）
（分担）研究報告書

アディポネクチン受容体結合蛋白の同定

（分担）研究者　植木　浩二郎　東京大学医学部附属病院
特任助教授

研究要旨：　アディポネクチン受容体の細胞内情報伝達の鍵となる結合蛋白としてPPALなどを同定した。

A．研究目的

我が国の死因の第一位を占める心血管疾患（心筋梗塞、脳梗塞等）の主要な原因は、肥満・糖尿病・高脂血症・高血圧が重複する所謂代謝症候群と考えられる。従って、メタボリックシンドロームの原因の解明とそれに立脚した根本的な予防法や治療法の確立が極めて重要である。我々はアディポネクチン受容体の同定が糖・脂質代謝の制御機構、メタボリックシンドローム・動脈硬化症の発症機構の解明に繋がるのみならず、新規の抗メタボリックシンドローム薬・抗動脈硬化薬の開発に繋がるものと考え、受容体の同定を試み、世界に先駆けて発見した(Nature 423:762,2003)。特許も取得している。本研究においては、受容体結合蛋白の同定と、それらに基づいた新規の革新的な抗メタボリックシンドローム・抗動脈硬化薬の開発も目指す。アディポネクチンの遺伝的・後天的欠乏は日本人における糖尿病・代謝症候群の主要な原因であり(Nature Medicine 7:941,2001;Nature Genetics 30: 221,2002: Nature Medicine 8:1288,2002:J. Biol. Chem. 279:30817, 2004)、病態生理的意義の解明と細胞内情報伝達経路の解明の必要性が極めて高いと言わざるを得ない。

B．研究方法

蛋白精製とLC/MSによる同定、yeast及びbacteriaを用いたtwo hybrid、プロテオミックスを用いた網羅的なアプローチの３つの手法を用いて、アディポネクチン受容体細胞内結合蛋白質を同定し、アディポネクチン作用における機能・役割を明らかにする。

E．結論

アディポネクチン受容体の細胞内情報伝達の鍵となる結合蛋白としてPPALなどを同定した。

F．健康危険情報

なし。

G．研究発表

1.　論文発表

Kubota N, et al. Pioglitazone ameliorates insulin resistance and diabetes by both adiponectin dependent and independent pathway. J Biol Chem. 281;8748-8755, 2006.

H．知的財産権の出願・登録状況（予定を含む。）

1.　特許取得

アディポネクチン受容体の細胞内情報伝達の鍵となる結合蛋白の特許申請準備中

2.　学会発表

（倫理面への配慮）
実験動物については「実験動物の飼養及び保管等に関する基準」（昭和55年3月27日 総理府告示）を遵守して実験に利用し、実験に伴う苦痛を最小限にするために適当な処置を行うこととする。本学に設置された動物実験施設の承認を得て許可された研究計画書に従い研究を実施する。動物実験の取り扱いに関しては倫理指針を遵守する。

C．研究結果

我々は、アディポネクチン受容体細胞内結合蛋白質のスクリーニングにより、アディポネクチン受容体AdipoR1・R2とそれぞれ特異的に結合する蛋白、共通に結合する蛋白を複数個同定した。これらのうちいくつかは構造のドメイン情報などにより、下流のAMPキナーゼ・PPARαの活性化に関与する候補分子と優先的に機能解析を進めている。特に興味深いものの１つは、AdipoR1と相対的に強く結合する蛋白である。チロシンリン酸化蛋白との結合部位(Phosphotyrosine binding domain)、リン酸化脂質との結合部位(Pleckstrin homology domain)、Aktとの結合部位、さらにLeucine zipper domainを持つシグナル分子として構造的に極めて興味深い蛋白(PPAL)である。

D．考察

遺伝子ノックアウトにより明らかとなったAdipoR1がAMPキナーゼの活性化とより強くリンクし、AdipoR2がPPARαの活性化により強くリンクしている、というAdipoR1とAdipoR2の役割分担のデータが、結合蛋白質の特異性によりを上手く説明出来る可能性があり、極めて興味深いデータと考えられる。

厚生労働科学研究費補助金（ヒトゲノム・再生医療等研究事業）
（分担）研究報告書

アディポネクチン情報伝達機構の解明

（分担）研究者　門脇　孝　東京大学医学部附属病院
糖尿病・代謝内科　教授

研究要旨：アディポネクチンとAdipoR1の結合がより強くAMPキナーゼの活性化を促進し、アディポネクチンとAdipoR2の結合がより強くPPARαを活性化することが明らかとなった。AdipoR1とより強く結合するPPALがアディポネクチンによるAMPキナーゼ活性化に重要な役割を果たすことを明らかにした。

A．研究目的

我が国の死因の第一位を占める心血管疾患（心筋梗塞、脳梗塞等）の主要な原因は、肥満・糖尿病・高脂血症・高血圧が重複する所謂メタボリックシンドロームと考えられる。従って、メタボリックシンドロームの原因の解明とそれに立脚した根本的な予防法や治療法の確立が極めて重要である。我々はアディポネクチン受容体の同定が糖・脂質代謝の制御機構、メタボリックシンドローム・動脈硬化症の発症機構の解明に繋がるのみならず、新規の抗メタボリックシンドローム薬・抗動脈硬化薬の開発に繋がるものと考え、受容体の同定を試み、世界に先駆けて発見した(Nature 423:762,2003)。特許も取得している。本研究においては、受容体結合蛋白の同定による細胞内情報伝達機構の解明と、それらに基づいた新規の革新的な抗代謝症候群・抗動脈硬化薬の開発も目指す。アディポネクチンの遺伝的・後天的欠乏は日本人における糖尿病・代謝症候群の主要な原因であり(Nature Medicine 7:941, 2001;Nature Genetics 30: 221,2002: Nature Medicine 8:1288,2002:J. Biol. Chem. 279:30817, 2004)、病態生理的意義の解明と細胞内情報伝達経路の解明の必要性が極めて高いと言わざるを得ない。

B．研究方法

分担研究によって同定されてくるアディポネクチン受容体細胞内結合蛋白質の、アディポネクチン作用における機能・役割を明らかにする。この目的に、興味深い候補として着目したPPALに

E．結論

AdipoR1とより強く結合するPPALがアディポネクチンによるAMPキナーゼ活性化に重要な役割を果たすことを明らかにした。AdipoR1・AdipoR2それぞれの特異的な結合タンパクがそれぞれの機能・役割・作用の違いを説明出来る可能性が示唆された。

F．健康危険情報

なし。

G．研究発表

1．論文発表

Tsuchida A, et al. Peroxisome proliferator-activated receptor (PPAR){alpha} activation increases adiponectin receptors and reduces obesity- related inflammation in adipose tissue: Comparison of activation of PPAR {alpha}, PPAR{gamma}, and their combination. Diabetes 54(12);3358-3370, 2005.

Kubota N, et al. Pioglitazone ameliorates insulin resistance and diabetes by both adiponectin dependent and independent pathway. J Biol Chem. 281;8748-8755, 2006.

Kadowaki T, et al. Adiponectin and adiponectin Receptors. Endocr Rev. 26(3); 439-451, 2005.

関して、モデル培養細胞を用いてsiRNA
を用いた遺伝子ノックダウンにより、
アディポネクチン作用に対する影響を
検討する。

(倫理面への配慮)
実験動物については「実験動物の飼養及び
保管等に関する基準」(昭和55年3月
27日 総理府告示)を遵守して実験に利用
し、実験に伴う苦痛を最小限にするため
に適当な処置を行うこととする。本学に
設置された動物実験施設の承認を得て許可
された研究計画書に従い研究を実施する。
動物実験の取り扱いに関しては倫理指針を
遵守する。

C．研究結果
我々は、アディポネクチン受容体細胞内
結合蛋白質のスクリーニングにより、アディ
ポネクチン受容体AdipoR1・R2とそれぞれ
特異的に結合する蛋白、共通に結合する蛋白
を複数個同定した。これらのうちいくつかは
構造のドメイン情報などにより、下流のAMP
キナーゼ・PPARαの活性化に関与する候補
分子として機能解析を進めた。興味深いもの
の１つは、AdipoR1とのみ結合する蛋白で
ある。チロシンリン酸化蛋白との結合部位
(Phosphotyrosine binding domain)、リン酸
化脂質との結合部位(Pleckstrin homology
domain)、Aktとの結合部位、さらにLeucine
zipper domainを持つシグナル分子として
構造的に極めて興味深い蛋白(PPAL)である。
RNAiを用いた実験から、この蛋白がAdipoR1
と結合してAMPキナーゼの活性化に重要な役割
を果たしているという極めて興味深いデータ
を得ている。

D．考察
本分担研究の結果は、他の分担研究者が
明らかにした、遺伝子ノックアウトにより
明らかとなったAdipoR1がAMPキナーゼの活性
化とより強くリンクし、AdipoR2がPPARαの
活性化により強くリンクしている、という
データと完全に合致するデータであり、結合
蛋白質の特異性によりAdipoR1とAdipoR2の
役割分担を上手く説明出来る可能性がある、
極めて興味深いデータと考えられる。今後、
構造情報や発現情報、ゲノム情報を最大限
利用して優先順位を設定し、順次機能解析を
進めていく予定である。

2. 学会発表

H．知的財産権の出願・登録状況
(予定を含む。)
1. 特許取得
アディポネクチン受容体の特異的結合
蛋白と作用・機能・役割の関係の特許
申請準備中

研究成果の刊行に関する一覧表レイアウト（参考）

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Tsuchida A, et al.	Peroxisome proliferator-activated receptor (PPAR) {alpha} activation increases adiponectin receptors and reduces obesity-related inflammation in adipose tissue: Comparison of activation of PPAR {alpha}, PPAR{gamma}, and their combination.	Diabetes	54	3358-3370	2005
Kubota N, et al.	Pioglitazone ameliorates insulin resistance and diabetes by both adiponectin dependent and independent path way.	J Biol Chem	281	8748-8755	2006
Kadowaki T, et al.	Adiponectin and adiponectin Receptors.	Endocr Rev	26	439-451	2005

Original Article

Peroxisome Proliferator–Activated Receptor (PPAR)α Activation Increases Adiponectin Receptors and Reduces Obesity-Related Inflammation in Adipose Tissue

Comparison of Activation of PPARα, PPARγ, and Their Combination

Atsushi Tsuchida,[1] Toshimasa Yamauchi,[1,2] Sato Takekawa,[1] Yusuke Hada,[1] Yusuke Ito,[1] Toshiyuki Maki,[1] and Takashi Kadowaki[1,2,3]

We examined the effects of activation of peroxisome proliferator–activated receptor (PPAR)α, PPARγ, and both of them in combination in obese diabetic KKAy mice and investigated the mechanisms by which they improve insulin sensitivity. PPARα activation by its agonist, Wy-14,643, as well as PPARγ activation by its agonist, rosiglitazone, markedly improved insulin sensitivity. Interestingly, dual activation of PPARα and -γ by a combination of Wy-14,643 and rosiglitazone showed increased efficacy. Adipocyte size in Wy-14,643–treated KKAy mice was much smaller than that of vehicle- or rosiglitazone-treated mice, suggesting that activation of PPARα prevents adipocyte hypertrophy. Moreover, Wy-14,643 treatment reduced inflammation and the expression of macrophage-specific genes in white adipose tissue (WAT). Importantly, Wy-14,643 treatment upregulated expression of the adiponectin receptor (AdipoR)-1 and AdipoR2 in WAT, which was decreased in WAT of KKAy mice compared with that in nondiabetic control mice. Furthermore, Wy-14,643 directly increased expression of AdipoRs and decreased monocyte chemoattractant protein-1 expression in adipocytes and macrophages. Rosiglitazone increased serum adiponectin concentrations and the ratio of high molecular weight multimers of adiponectin to total adiponectin. A combination of rosiglitazone and Wy-14,643 increased both serum adiponectin concentrations and AdipoR expression in WAT. These data suggest that PPARα activation prevents inflammation in WAT and that dual activation of PPARα and -γ enhances the action of adiponectin by increasing both adiponectin and AdipoRs, which can result in the amelioration of obesity-induced insulin resistance. *Diabetes* 54:3358–3370, 2005

Peroxisome proliferator–activated receptor (PPAR)α and -γ are ligand-activated transcription factors and members of the nuclear hormone receptor superfamily that regulate the metabolism of glucose and lipids (1–6). PPARγ is one of the key regulators of glucose homeostasis, and the molecular mechanisms concerning how the activation of PPARγ improves insulin sensitivity have been well investigated. Activation of PPARγ by agonists such as thiazolidinediones (TZDs) stimulates lipid storage in adipocytes, thereby reducing lipotoxicity in liver and skeletal muscle (7). In addition, PPARγ activation increases small adipocytes, thereby increasing the insulin-sensitizing hormone adiponectin and reducing resistin and tumor necrosis factor (TNF)-α, which induce insulin resistance (8,9). However, PPARγ agonists are associated with body weight gain, which is a clinical drawback for treatment of type 2 diabetic patients. In contrast, it has been previously reported that PPARα agonists prevent the development of obesity-induced insulin resistance in rodents without inducing body weight gain (10–12); however, the mechanisms by which the activation of PPARα improves insulin resistance are not fully understood.

Recently, it has been reported that chronic inflammation in white adipose tissue (WAT) by macrophage infiltration may cause whole-body insulin resistance in obese diabetic animals (13,14). Activated macrophages that infiltrate into WAT secrete cytokines that can impair adipocyte insulin sensitivity. Adipocytes stimulated by proinflammatory cytokines secrete chemokines that can contribute to macrophage infiltration. This vicious cycle impairs adipocyte insulin signaling and may eventually cause systemic insulin resistance (13,14). Moreover, inflammatory markers such as C-reactive protein are associated with insulin resistance, adiposity, and type 2 diabetes in human subjects (15–17). Therefore, it has become important to investigate the mechanisms of insulin-sensitizing drugs by focusing on the regulation of inflammation.

In this study, we examined the effects of activation of

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A.T. and T.Y. contributed equally to this work.

AdipoR, adiponectin receptor; BAT, brown adipose tissue; DMEMH, Dulbecco's modified high-glucose Eagle's medium; HMW, high molecular weight; MCP, monocyte chemotactic protein; PDK4, pyruvate dehydrogenase kinase isozyme 4; PPAR, peroxisome proliferator–activated receptor; TNF, tumor necrosis factor; TZD, thiazolidinedione; UCP, uncoupling protein; WAT, white adipose tissue.

PPARα, PPARγ, and both in combination in obese diabetic KKAy mice, and we investigated the mechanisms by which they improve insulin resistance, especially in WAT. The results indicate that PPARα activation prevents infiltration of macrophages into WAT, thereby ameliorating inflammation of WAT, which can result in improvement of obesity-induced insulin resistance. We also demonstrate that dual activation of PPARα and -γ enhances the action of adiponectin in WAT by increasing both adiponectin and the expression of its receptors.

RESEARCH DESIGN AND METHODS

Rosiglitazone was synthesized as described elsewhere (18). Wy-14,643 was purchased from Biomol Research Laboratories (Plymouth Meeting, PA). All materials were obtained from sources described previously (8,9,19).

We purchased 6-week-old male KKAy mice and age-matched KK mice from Nippon CLEA (Shizuoka, Japan). KKAy mice are an obese diabetic model in which the Ay mutation is introduced onto a KK strain background. Therefore, we used KK mice as nondiabetic controls. Mice were housed singly and maintained on a 12-h light/dark cycle. The high-fat diet consisted of 32% (wt/wt) fat as described previously (20). KKAy mice were given the high-fat diet, and KK mice were given normal chow. High-fat feeding was begun 1 week before rosiglitazone or Wy-14,643 administration. Rosiglitazone or Wy-14,643 was given as a 0.01 and 0.05% food admixture, respectively. These doses were chosen because they have been shown to be effective therapeutic doses in diabetic mice (21–24). The same amount of food was given to the pair-fed group of KKAy mice as that fed to the Wy-14,643–treated group of KKAy mice. The animal care procedures and methods were approved by the animal care committee of the University of Tokyo.

Blood sample assays and in vivo glucose homeostasis. The glucose tolerance and insulin tolerance tests were carried out according to previously described methods (9). Plasma glucose, serum free fatty acid, and triglyceride levels were determined by a glucose test, nonesterified fatty acid-C test, and triglyceride L-type (Wako Pure Chemical Industries, Osaka, Japan), respectively. Plasma insulin was measured by an insulin immunoassay (Shibayagi, Gunma, Japan). Plasma leptin and adiponectin levels were determined by a Quintikine M kit (R&D Systems, Minneapolis, MN) and mouse adiponectin immunoassay kit (Otsuka Pharmaceutical, Tokushima, Japan), respectively (20). Detection of multimer species of adiponectin was conducted as described previously (25). In brief, 0.7 μl of serum was subjected to 2–15% SDS-PAGE under nonreducing and non–heat-denaturing conditions. Adiponectin was detected using anti–globular domain antiserum obtained by immunizing rabbits with mouse recombinant adiponectin globular domain produced in *Escherichia coli* (25).

Histological analysis of adipose tissue. Epididymal adipose tissue was removed from each animal, fixed in 10% formaldehyde/PBS, and maintained at 4°C until use. Fixed specimens were dehydrated, embedded in tissue-freezing medium (Tissue-Tek OCT compound; Miles, Elkhart, IN), and frozen in dry ice and acetone. WAT was cut into 10-μm sections, and the sections were mounted on silanized slides. The adipose tissue was stained with hematoxylin and eosin (7) or anti-mouse F4/80 antibody (Serotec, Raleigh, NC) (13).

Quantitative analysis by real-time PCR. Total RNA was prepared from cells or tissues with TRIzol (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. A real-time PCR method was used to quantify the AdipoRs mRNAs (19). The primer sets and the probes for mAdipoR1 and -R2 were as follows: the forward primer for mAdipoR1 was acgttggagagtcatcccg tat, the reverse primer ctctgtgtggatgcggaagat, and the probe cctgctacatggcca cagaccacct; the forward primer for mAdipoR2 was tcccaggaagatgaagggtttat, the reverse primer ttccattcgttcgatagcatga, and the probe atgtccccgctcctacagg ccc. For quantification of the other genes, we used a set of predesigned primers and a probe for each gene (Assay on Demand; Applied Biosystems, Foster City, CA). The relative amount of each transcript was normalized to the amount of β-actin transcript in the same cDNA (19).

Isolation of adipocytes and stromal-vascular cells. Isolation of adipocytes and stromal-vascular cells from adipose tissues were performed as described previously, with slight modification (13). Epididymal adipose tissues were isolated from mice and were minced into fine pieces. Minced tissues were incubated in Dulbecco's modified Eagle's medium supplemented with 5 mg/ml collagenase (Sigma, St. Louis, MO) and 2.5% BSA (Sigma) at 37°C for 40–45 min. Digested samples were passed through a sterile 250-μm nylon mesh and centrifuged. The pellet cells and the floating cells were washed twice with PBS and collected as the stromal-vascular cells and adipocytes, respectively. In the experiments of direct action of PPAR agonists on primary adipocytes and stromal-vascular cells, 1×10^6 isolated adipocytes were seeded onto 24-well plates in Dulbecco's modified high-glucose Eagle's medium (DMEMH) supplemented with 20% charcoal-treated FCS, and 1×10^5 isolated stromal-vascular cells were seeded onto 96-well plates in DMEMH supplemented with 20% charcoal-treated FCS and mouse granulocyte colony-stimulating factor (R&D Systems). Then, the cells were incubated with 3 μmol/l Wy-14,643 or 0.3 μmol/l rosiglitazone for 24 h and then harvested to isolate total RNA.

Studies with 3T3-L1 adipocytes and peritoneal macrophages. Mouse 3T3-L1 cells were grown in DMEMH supplemented with 10% FCS. Induction of adipogenic differentiation was carried out according to a method described previously (8). By day 8, 3T3-L1 adipocytes were incubated with 30 μmol/l Wy-14,643 or 0.3 μmol/l rosiglitazone in DMEMH supplemented with 1% BSA for 18 h, and then 1 ng/ml TNF-α was added. The cells were harvested 6 h after TNF-α addition to isolate total RNA. Peritoneal macrophages were isolated as previously described (26). In brief, macrophages were isolated 4 days after intraperitoneal injection of 3 ml thioglycolate medium (Sigma) to male C57BL/6j mice. Then, 1,000,000 cells were plated onto 24-well plates in RPMI-1640/10% FBS (vol/vol) and incubated for 4 h. Then, the macrophages were incubated with 30 μmol/l Wy-14,643, 100 μmol/l fenofibrate, or 0.3 μmol/l rosiglitazone in the presence of 0.1 μg/ml lipopolysaccharide for 24 h and then harvested to isolate total RNA. The concentrations of the compounds in the in vitro cell culture experiments were chosen according to previous studies, and they were comparable to those to which PPARα in animal tissues were exposed in previous in vivo studies (8,10–12,27,28).

Statistical analysis. Data are the means ± SE. Student's *t* test was used for statistical comparison. $P < 0.05$ was considered statistically significant.

RESULTS

A PPARα agonist improves insulin resistance in KKAy mice, and a combination of a PPARα agonist and a PPARγ agonist enhances the antidiabetic effects of the PPARγ agonist. To examine the antidiabetic effects of a dual activation of PPARα and -γ in comparison with a single activation of each alone, we treated obese diabetic KKAy mice with a PPARγ agonist, rosiglitazone, a PPARα agonist, Wy-14,643, or a combination of the two for 8 weeks. We then examined the glucose and lipid metabolism and insulin sensitivity of these mice. Because it has been previously reported that PPARα agonists reduce food intake in rodents (28), pair-fed control mice were given daily the same amount of food as that consumed by Wy-14,643–treated mice (Fig. 1*F*). As shown in Fig. 1, Wy-14,643 treatment significantly ameliorated hyperglycemia (Fig. 1*A*), hyperinsulinemia (Fig. 1*B*), and hyperlipidemia (Fig. 1*C* and *D*) compared with ad libitum–fed vehicle-treated KKAy mice. Because these effects of Wy-14,643 were significant even in comparison with pair-fed mice (Fig. 1*A–D*), it is likely that Wy-14,643 actually exerted its antidiabetic effects via a mechanism other than decreased food intake. Blood glucose (Fig. 1*A*) and plasma insulin levels (Fig. 1*B*) in the rosiglitazone-treated mice were significantly lower than those in the ad libitum–fed vehicle-treated mice. However, the serum lipid levels of the rosiglitazone-treated mice were significantly higher than those of the Wy-14,643–treated mice (Fig. 1*C* and *D*). Interestingly, a combination of rosiglitazone and Wy-14,643 completely normalized the hyperglycemia (Fig. 1*A*), hyperinsulinemia (Fig. 1*B*), and hyperlipidemia (Fig. 1*C* and *D*) observed in KKAy mice, and, in particular, the blood glucose (Fig. 1*A*) and lipid levels (Fig. 1*C* and *D*) in the combined rosiglitazone- and Wy-14,643–treated mice were significantly lower than those in the rosiglitazone-treated mice. Furthermore, serum lipid levels in the combined rosiglitazone- and Wy-14,643–treated mice were significantly lower than those in the Wy-14,643–treated mice (Fig. 1*C* and *D*). Although body weight was slightly lower in the Wy-14,643–treated mice ($P = 0.093$) than in the pair-fed vehicle-treated mice, the difference was not statistically significant (Fig. 1*E*).

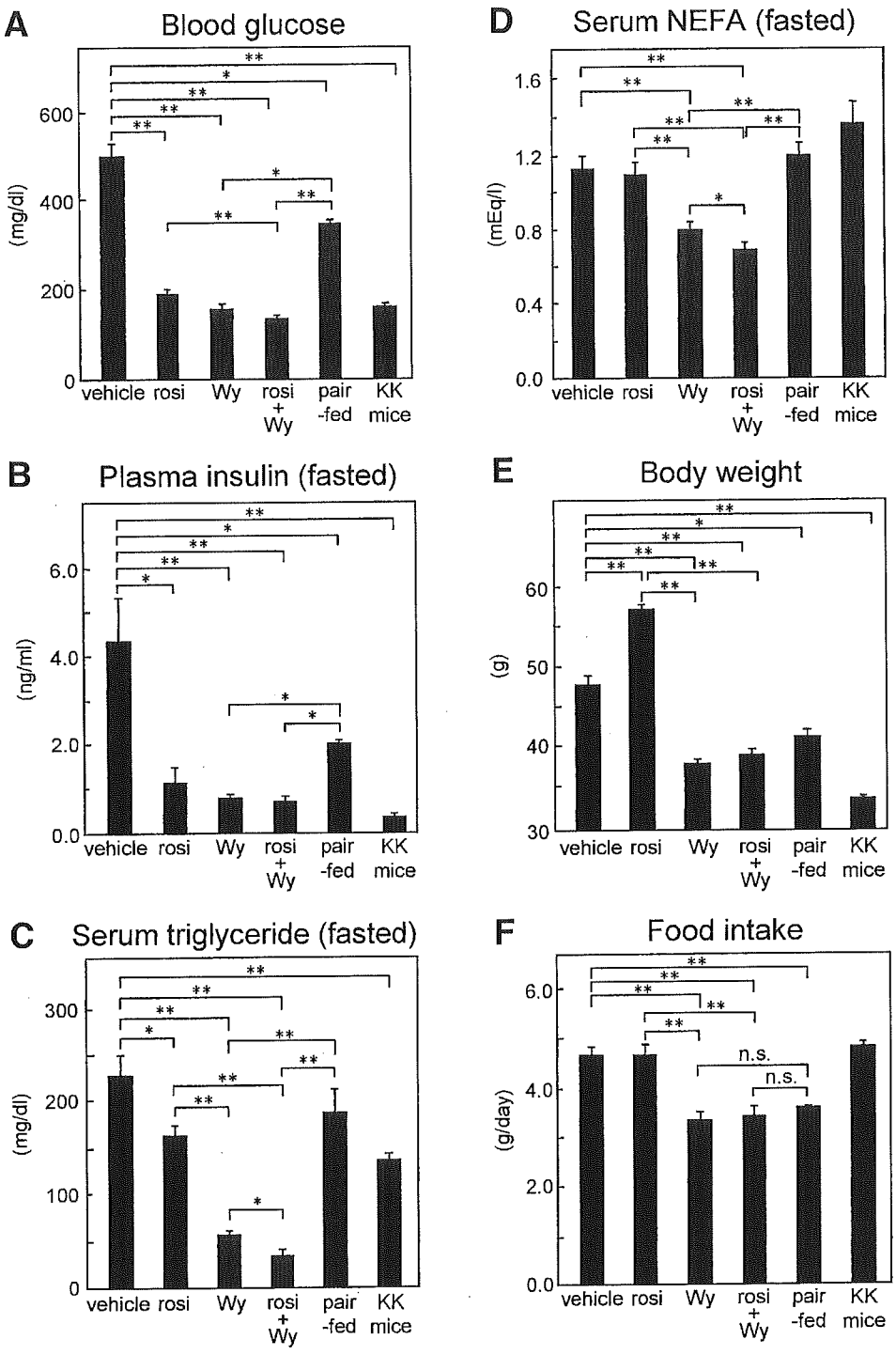

FIG. 1. Effects of rosiglitazone, Wy-14,643, or both rosiglitazone and Wy-14,643 treatment for 8 weeks on serum parameters and body weight in KKAy mice. Panels show blood glucose (*A*), fasting plasma insulin (*B*), fasting serum triglycerides (*C*), fasting NEFA (*D*), body weight (*E*), and food intake (*F*) of male KKAy mice treated with 0.01% rosiglitazone (rosi), 0.05% Wy-14,643 (Wy), or both 0.01% rosiglitazone and 0.05% Wy-14,643 (rosi+Wy) as food admixture for 8 weeks while on the high-fat diet. The same amount of food was given to the pair-fed group as to mice treated with Wy-14,643. Age-matched wild-type KK mice given normal chow were used as normal controls. Fasting parameters were measured after a 24-h fast. Each bar represents the means ± SE (n = 4 for pair-fed group, n = 6 for other groups). *P < 0.05; **P < 0.01. NEFA, nonesterified fatty acid; n.s., not significant.

We next examined the effects of PPARα and -γ activation on the improvement of insulin resistance in more detail, using a glucose tolerance test and insulin tolerance test. Blood glucose levels during both tests in Wy-14,643–treated mice were significantly lower than those in pair-fed vehicle-treated mice (Fig. 2*A* and *C*), suggesting that Wy-14,643 treatment ameliorated insulin resistance. Again, the combination of rosiglitazone and Wy-14,643 ameliorated insulin resistance more effectively than rosiglitazone or Wy-14,643 alone (Fig. 2*B* and *C*).

Adipocyte hypertrophy is prevented by a PPARα agonist. It has been reported that PPARα agonists prevent high-fat diet–induced obesity (10). Thus, we measured epididymal and subcutaneous WAT and intrascapular brown adipose tissue (BAT) weights. Wy-14,643 decreased both WAT and BAT weights compared with pair-fed mice (Fig. 3*A*–*C*), suggesting that PPARα activation prevents obesity. The combination of rosiglitazone and Wy-14,643 decreased only epididymal WAT weights compared with pair-fed mice (Fig. 3*A*). In contrast, rosiglitazone increased subcutaneous WAT and BAT weights compared with vehicle (Fig. 3*B* and *C*).

We have previously shown that PPARγ activation by its agonist or a moderate reduction of PPARγ activity prevents adipocyte hypertrophy, which results in an improvement in insulin resistance (7). We next attempted to clarify

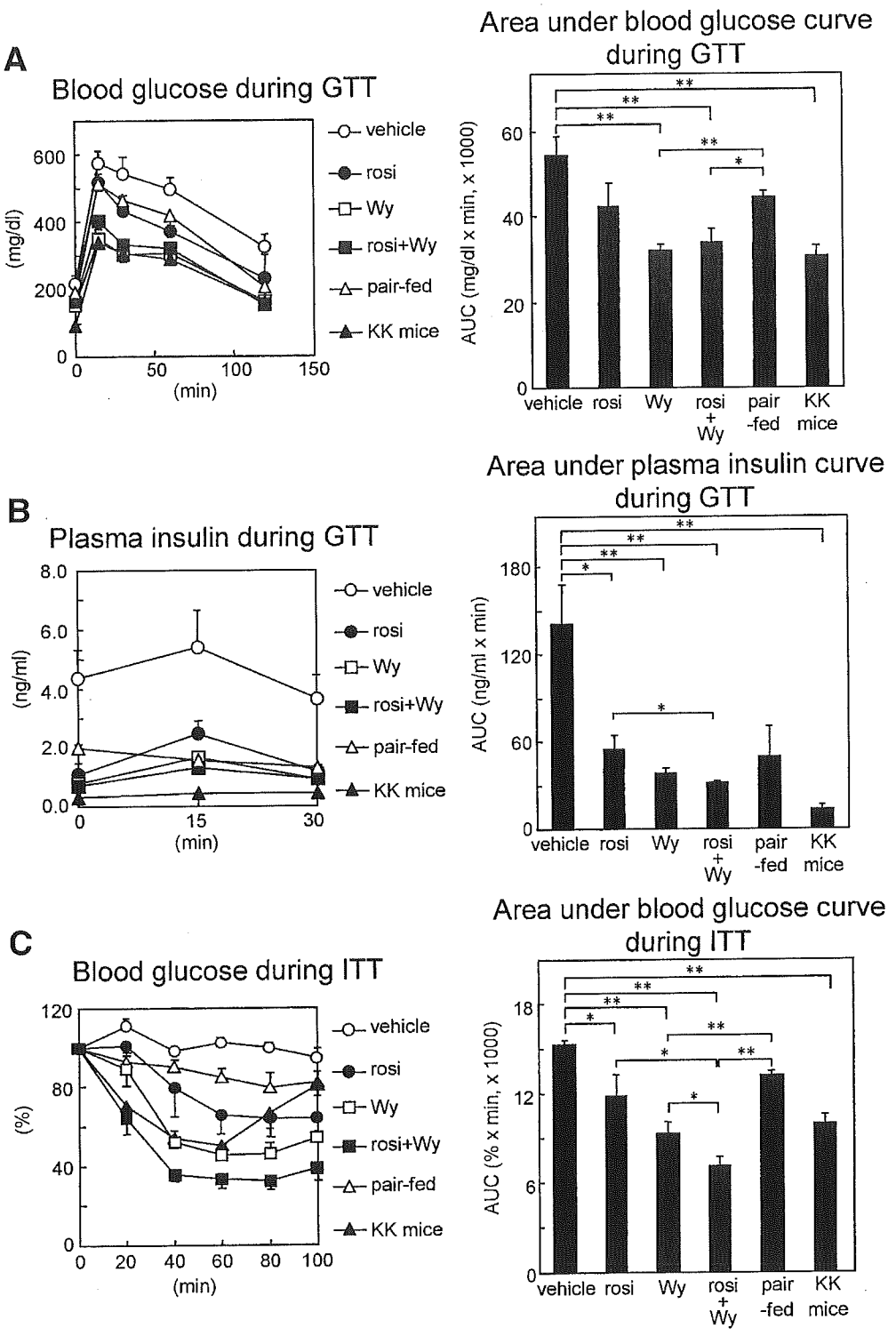

FIG. 2. Effects of rosiglitazone, Wy-14,643, or both rosiglitazone and Wy-14,643 treatment for 8 weeks on glucose tolerance and insulin sensitivity in KKAy mice. We measured blood glucose (*A*) and plasma insulin (*B*) during oral glucose tolerance tests (GTTs) and blood glucose during insulin tolerance tests (ITTs) (*C*) of male KKAy mice treated with 0.01% rosiglitazone (rosi), 0.05% Wy-14,643 (Wy), or both 0.01% rosiglitazone and 0.05% Wy-14,643 (rosi+Wy) as a food admixture for 8 weeks while on the high-fat diet. The same amount of food was given to the pair-fed group as to mice treated with Wy-14,643. Age-matched wild-type KK mice given normal chow were used as normal controls. Oral glucose tolerance tests were performed by oral gavage of 0.75 g/kg body wt glucose after 24 h fasting followed by blood sampling at the indicated time. Insulin tolerance tests were performed by 1.5 units/kg body wt i.p. insulin followed by blood sampling at the indicated time. Each bar represents the means ± SE (n = 4 for pair-fed group, n = 6 for other groups). ○, vehicle; ●, rosiglitazone; □, Wy-14,643; ■, both rosiglitazone and Wy-14,643; △, pair-fed; ▲, KK mice. *P < 0.05; **P < 0.01.

whether adipocyte hypertrophy and increased fat pad weight are suppressed by PPARα activation. The size of the adipose cells in epididymal WAT was increased in KKAy mice (Fig. 4*A*, *upper left*) compared with the wild-type control KK mice (Fig. 4*A*, *lower right*). Very interestingly, the size of the adipose cells in epididymal WAT from Wy-14,643–treated mice (Fig. 4*A*, *upper right*) was dramatically decreased compared with that from pair-fed mice (Fig. 4*A*, *lower middle*) and was comparable to that in the wild-type control KK mice (Fig. 4*A*, *lower right*). Although rosiglitazone treatment (Fig. 4*A*, *upper middle*) increased the ratio of small adipocytes in epididymal WAT compared with vehicle treatment (Fig. 4*A*, *upper left*), the changes were moderate compared with Wy-14,643 treatment (Fig. 4*A*, *upper right*). The size of the adipose cells in epididymal WAT from mice treated with a combination of rosiglitazone and Wy-14,643 (Fig. 4*A*, *lower left*) was also smaller than that from pair-fed mice (Fig. 4*A*, *lower middle*). The size of the adipose cells in subcutaneous WAT from Wy-14,643–treated mice was also decreased compared with that from pair-fed mice (Fig. 4*B*). There were small nucleated cells and macrophage-specific antigen F4/80–

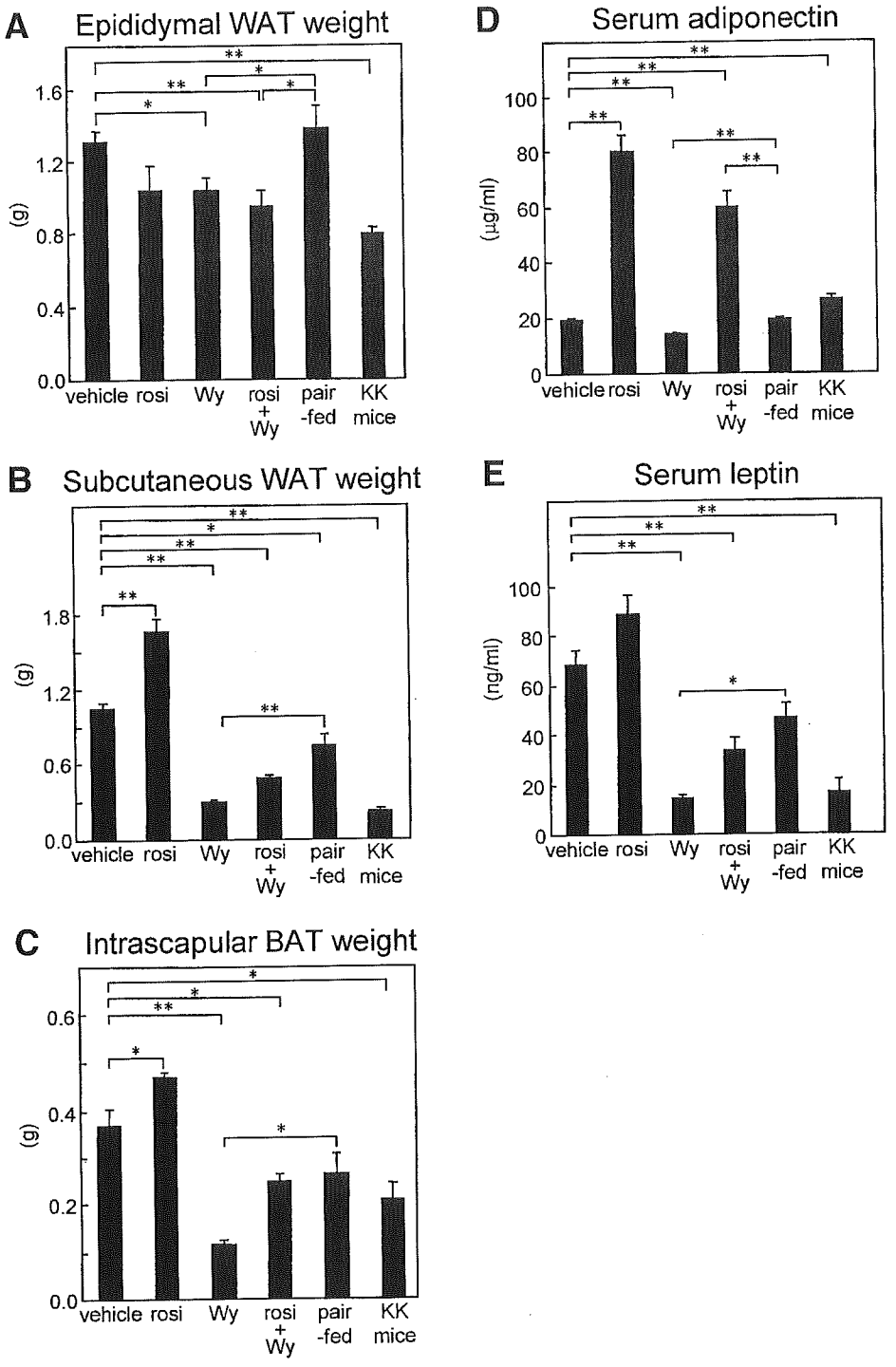

FIG. 3. Effects of rosiglitazone, Wy-14,643, or both rosiglitazone and Wy-14,643 treatment for 8 weeks on fat pad weight and serum adipokines in KKAy mice. Panels show epididymal WAT weight (*A*), subcutaneous WAT weight (*B*), intrascapular BAT weight (*C*), serum adiponectin levels (*D*), and serum leptin levels (*E*) of male KKAy mice treated with 0.01% rosiglitazone (rosi), 0.05% Wy-14,643 (Wy), or both 0.01% rosiglitazone and 0.05% Wy-14,643 (rosi+Wy) as a food admixture for 8 weeks while on the high-fat diet. The same amount of food was given to the pair-fed group as to mice treated with Wy-14,643. Age-matched wild-type KK mice given normal chow were used as normal controls. Each bar represents the means ± SE (n = 4 for pair-fed group, n = 6 for other groups). *$P < 0.05$; **$P < 0.01$.

expressing cells in the interstitial spaces between adipocytes in WAT of vehicle-treated KKAy mice (Fig. 4*C*). In contrast, there were almost no such cells in the WAT of Wy-14,643–treated mice (Fig. 4*C*), suggesting that macrophage infiltration to WAT may be suppressed by Wy-14,643 treatment, whereas the effect of rosiglitazone treatment seemed to be faint. We obtained similar results with BAT (Fig. 4*D*), except that the size of the adipocytes in BAT from rosiglitazone-treated mice (Fig. 4*D*, *upper middle*) was larger than that in vehicle-treated mice (Fig. 4*D*, *upper left*).

We next studied whether the levels of expression of molecules secreted from WAT that regulate insulin sensitivity were changed by PPARα activation. As reported previously (9), serum adiponectin levels in rosiglitazone-treated mice were higher by fourfold than those in vehicle-treated mice (Fig. 3*D*). In contrast, serum adiponectin levels in Wy-14,643–treated mice were slightly lower than those in pair-fed mice, suggesting that the improvement in insulin resistance by Wy-14,643 was not caused by increased gene expression or secretion of adiponectin (Fig. 3*D*). The combination of rosiglitazone and Wy-14,643 increased serum adiponectin levels approximately threefold above the vehicle (Fig. 3*D*). Serum leptin levels in KKAy mice were increased by overexpression of agouti compared with those in wild-type KK mice (Fig. 3*E*). Wy-14,643

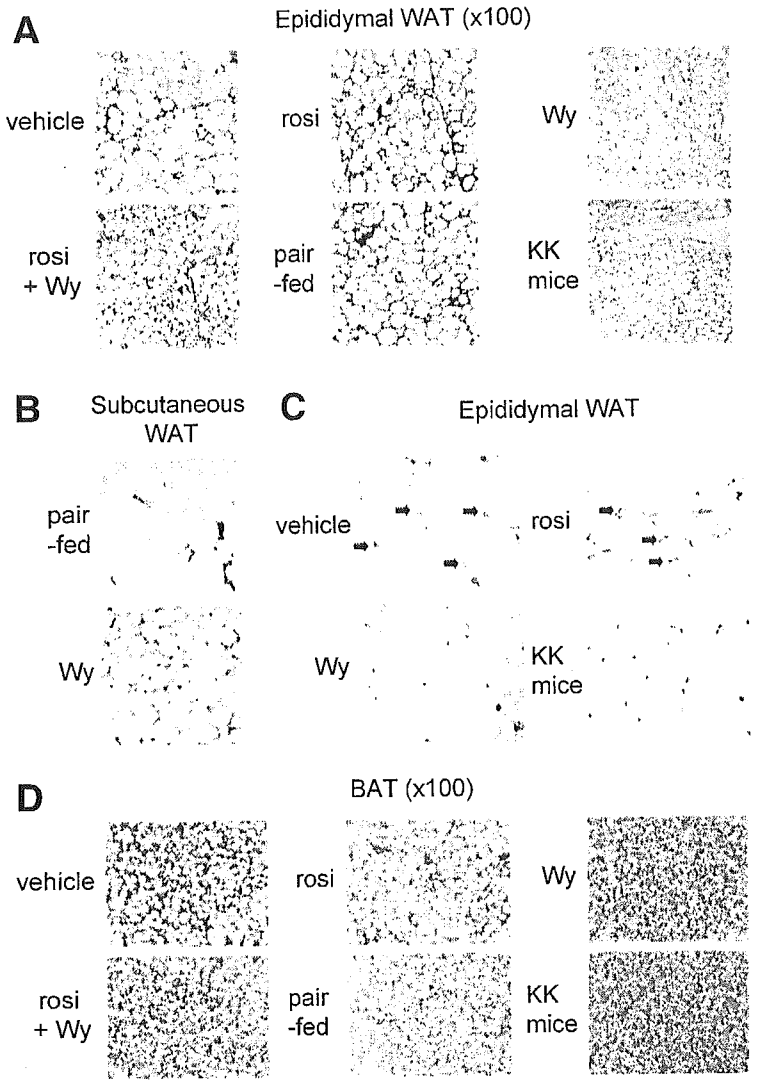

FIG. 4. Histological analyses of WAT and BAT from KKAy mice treated with rosiglitazone, Wy-14,643, or both rosiglitazone and Wy-14,643 for 8 weeks. Panels show the sections of epididymal WAT (*A* and *C*), subcutaneous WAT (*B*), and BAT (*D*), which were stained with hematoxylin and eosin (*A*, *B*, and *D*) or anti-F4/80 antibody (*C*), of male KKAy mice were treated with 0.01% rosiglitazone (rosi), 0.05% Wy-14,643 (Wy), or both 0.01% rosiglitazone and 0.05% Wy-14,643 (rosi+Wy) as a food admixture for 8 weeks while on the high-fat diet. The same amount of food was given to the pair-fed group as to mice treated with Wy-14,643. Age-matched wild-type KK mice given normal chow were used as normal controls. Arrows indicate F4/80-expressing cells.

treatment decreased the serum leptin levels to levels comparable to wild-type KK mice (Fig. 3*E*). Although rosiglitazone treatment slightly ($P = 0.057$) increased serum leptin levels, combined rosiglitazone and Wy-14,643 treatment decreased them compared with vehicle (Fig. 3*E*).

A PPARα agonist increases molecules involved in fatty acid combustion in liver and BAT. PPARα is abundantly expressed in liver and BAT in rodents, and PPARα activation induces fatty acid combustion (29,30). Thus, we examined the gene expression of molecules involved in fatty acid oxidation and lipolysis, using quantitative PCR analyses. As shown in Fig. 5*B* and *C*, the expression of uncoupling protein (UCP)-1 and β3-adrenergic receptor in BAT were decreased in vehicle-treated KKAy mice compared with wild-type KK mice, consistent with the adipocyte hypertrophy observed in KKAy mice (Fig. 5*C*). Wy-14,643 treatment increased the expression of UCP2 in liver (Fig. 5*A*) and the expression of UCP1 and β3-adrenergic receptor in BAT (Fig. 5*B* and *C*). In contrast, rosiglitazone treatment decreased the expression of UCP1 and β3-adrenergic receptor in BAT compared with vehicle (Fig. 5*B* and *C*). Combined treatment with rosiglitazone and Wy-14,643 increased the expression of UCP2 (Fig. 5*A*), but it did not affect the expression of UCP1 and β3-adrenergic receptor in BAT compared with vehicle (Fig. 5*B* and *C*). Taken together, these findings suggest that the induction of molecules involved in fatty acid combustion in liver and BAT is, at least in part, responsible for the prevention of adipocyte hypertrophy by PPARα activation.

A PPARα agonist suppresses inflammation in WAT. Recently, it was proposed that obesity-related insulin resistance may be a chronic inflammatory disease initiated in adipose tissue (13,14). Although it has been reported that rosiglitazone suppressed the expression of inflammation genes in WAT of obese diabetic *ob/ob* mice (14), the effects of PPARα agonists on inflammation in WAT have not yet been studied in detail. We studied whether Wy-14,643 suppressed the expression of inflammatory genes in WAT. As shown in Fig. 5*D–F*, the expression of TNF-α, monocyte chemotactic protein (MCP)-1, and macrophage antigen-1 in WAT of vehicle-treated KKAy mice were significantly increased compared with wild-type KK mice, suggesting that the accumulation of macrophages and inflammatory responses were induced in WAT of KKAy mice. Interestingly, Wy-14,643 treatment suppressed the increased expression of TNF-α, MCP-1, and macrophage antigen-1 in WAT compared with vehicle (Fig. 5*D–F*), which was consistent with the suppression of macrophage infiltration into WAT by Wy-14,643 treatment (Fig. 4*C*). Rosiglitazone treatment partially suppressed the expression of MCP-1 in WAT (Fig. 5*F*), but it did not affect the expression of TNF-α and macrophage antigen-1 compared

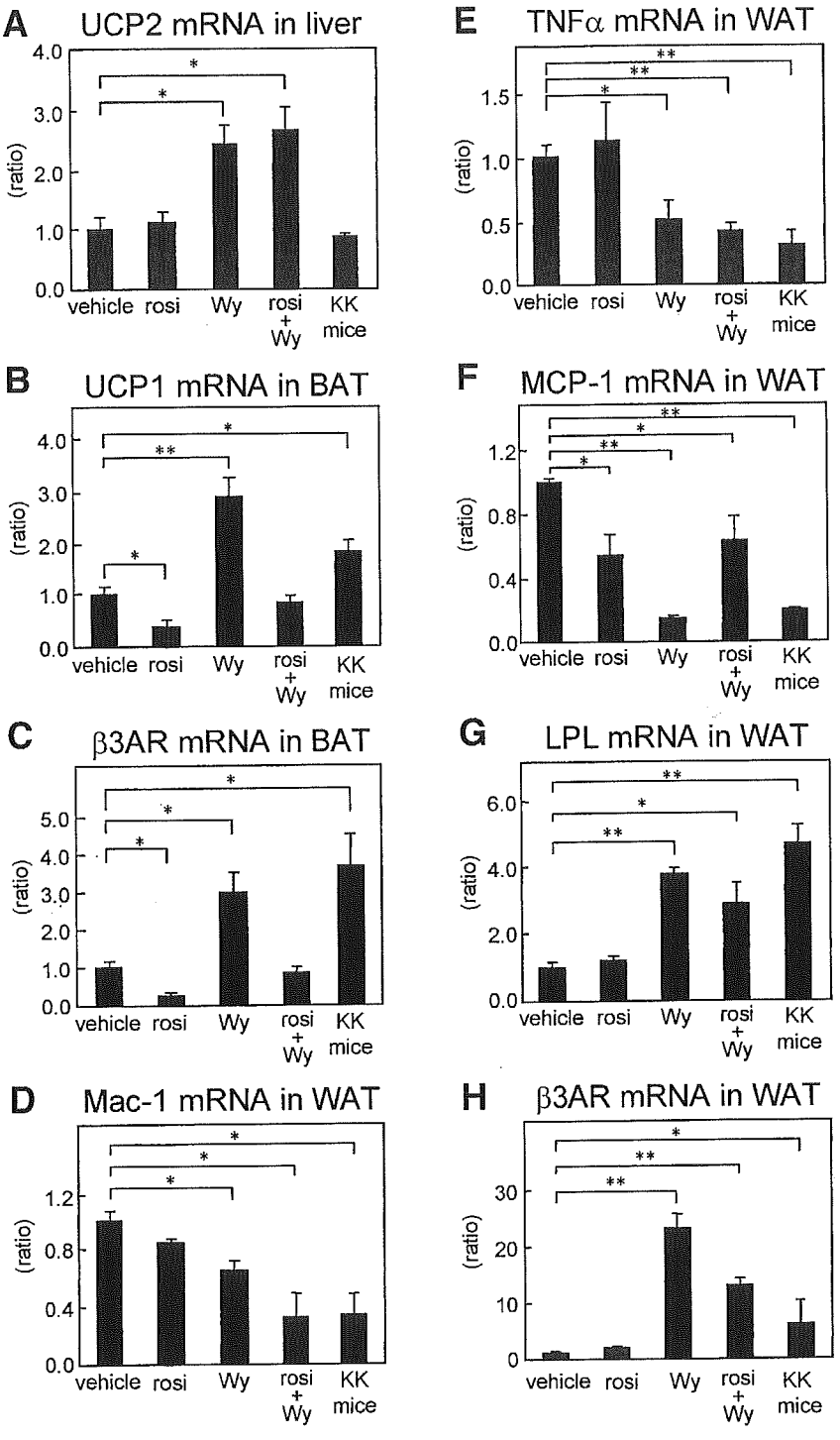

FIG. 5. Effects of rosiglitazone, Wy-14,643, or both rosiglitazone and Wy-14,643 treatment for 8 weeks on expression of molecules involving fatty acid combustion and inflammation in liver, BAT, and WAT from KKAy mice. Panels show amounts of mRNAs of UCP2 in liver (*A*); UCP1 (*B*) and β3-adrenergic receptor (*C*) in BAT; and macrophage antigen (Mac)-1 (*D*), TNF-α (*E*), MCP-1 (*F*), lipoprotein lipase (*G*), and β3-adrenergic receptor (β3AR) (*H*) in epididymal WAT of male KKAy mice treated with 0.01% rosiglitazone (rosi), 0.05% Wy-14,643 (Wy), or both 0.01% rosiglitazone and 0.05% Wy-14,643 (rosi+Wy) as food admixture for 8 weeks while on the high-fat diet. Age-matched wild-type KK mice given normal chow were used as normal controls. Amounts of the mRNAs of molecules indicated above were quantified by a real-time PCR method as described in RESEARCH DESIGN AND METHODS. The relative amount of each transcript was normalized to the amount of β-actin transcript in the same cDNA. The results are expressed as the ratio of the value of vehicle-treated KKAy mice. Each bar represents the means ± SE (n = 3). *P < 0.05; **P < 0.01.

with vehicle (Fig. 5*D* and *E*). Combined rosiglitazone and Wy-14,643 treatment, as well as Wy-14,643 treatment, suppressed the expression of these genes compared with vehicle (Fig. 5*D–F*).

It has been reported that MCP-1 decreased the expression of genes involved in adipocyte function and attenuated insulin sensitivity in cultured adipocytes (31). Thus, we examined the effects of PPARα activation on the expression of lipoprotein lipase and β3-adrenergic receptor in WAT. The expression of lipoprotein lipase and β3-adrenergic receptor were decreased in WAT of vehicle-treated KKAy mice compared with wild-type KK mice (Fig. 5*G* and *H*). Wy-14,643 treatment increased the expression of lipoprotein lipase and β3-adrenergic receptor in WAT, whereas rosiglitazone treatment showed no effect (Fig. 5*G* and *H*). Taken together, these data suggest that Wy-14,643 suppressed inflammation in WAT and normalized gene expression, which were dysregulated by obesity-associated adipocyte hypertrophy and inflammation.

A PPARα agonist increases the expression of adiponectin receptors (AdipoRs) in WAT, whereas a PPARγ agonist increases the ratio of high molecular weight multimers of adiponectin to total adiponectin. We previously reported that AdipoR1 and -2 are downregulated in WAT, BAT, and skeletal muscles in obese diabetic *ob/ob* mice, which are correlated with decreased adiponec-

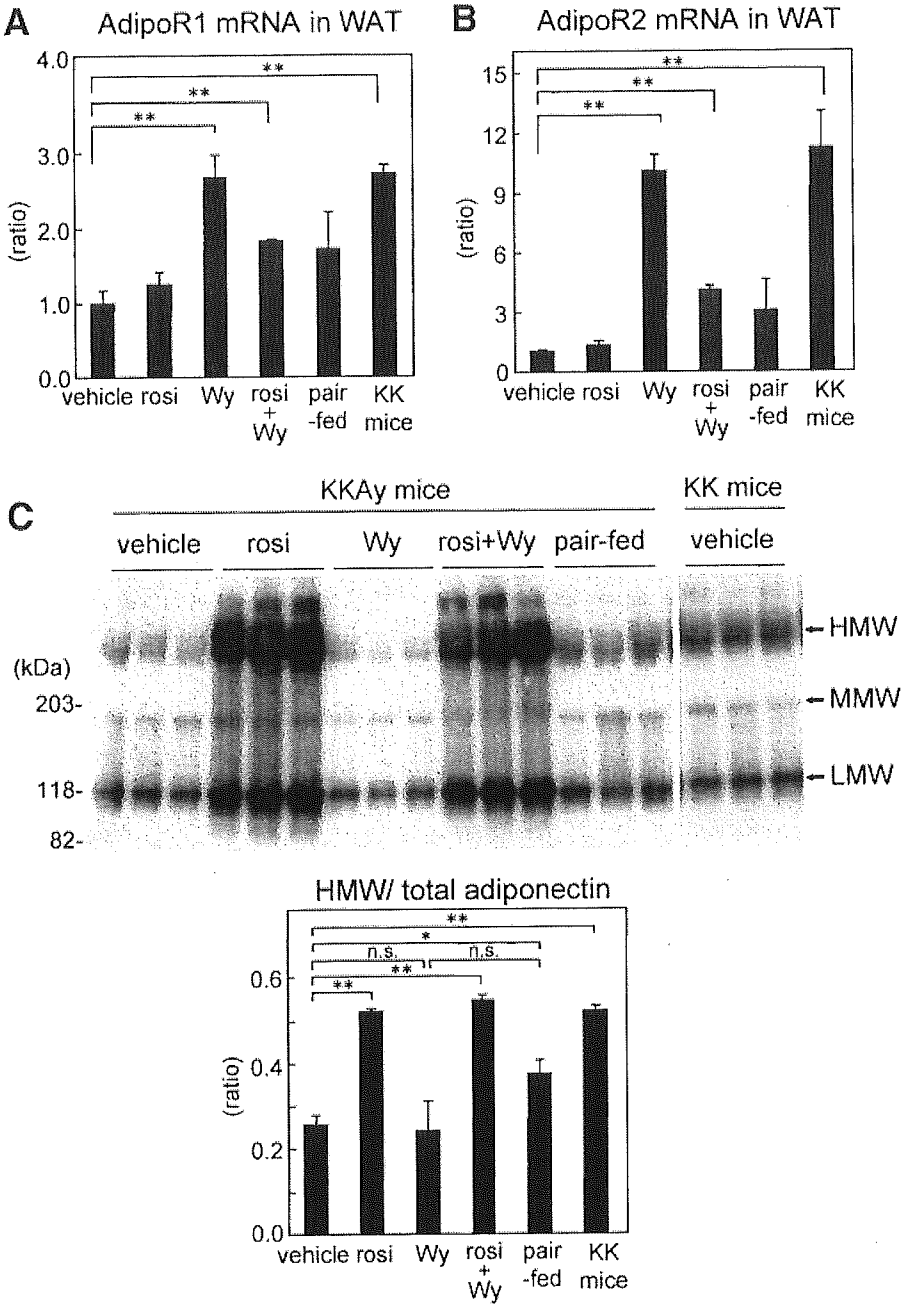

FIG. 6. Effects of rosiglitazone, Wy-14,643, or both rosiglitazone and Wy-14,643 treatment for 8 weeks on expression of AdipoR1/R2 in WAT and multimer forms of serum adiponectin in KKAy mice. Panels show amounts of mRNAs of AdipoR1 (*A*) and AdipoR2 (*B*) in epididymal WAT of male KKAy mice treated with 0.01% rosiglitazone (rosi), 0.05% Wy-14,643 (Wy), or both 0.01% rosiglitazone and 0.05% Wy-14,643 (rosi+Wy) as food admixture for 8 weeks while on the high-fat diet. The same amount of food was given to the pair-fed group as to mice treated with Wy-14,643. Age-matched wild-type KK mice given normal chow were used as normal controls. Amounts of the mRNAs of AdipoR1/R2 were quantified by a real-time PCR method as described in RESEARCH DESIGN AND METHODS. The relative amount of each transcript was normalized to the amount of β-actin transcript in the same cDNA. The results are expressed as the ratio of the value of vehicle-treated KKAy mice. Serum of KKAy mice treated with compounds mentioned above was subjected to SDS-PAGE under nonreducing, non–heat-denaturing conditions, and multimer forms of adiponectin were detected, using anti-adiponectin antibody (*C*). *D*: The quantitative results of upper panels. Each bar represents the means ± SE ($n = 3$). *$P < 0.05$; **$P < 0.01$. LMW, low molecular weight; n.s., not significant.

tin sensitivity (32). As shown in Fig. 6*A* and *B*, the expression of AdipoR1 and -2 was decreased in WAT of vehicle-treated KKAy mice compared with wild-type KK mice. Wy-14,643 treatment almost completely reversed the decrease in AdipoR1 and -2 expression in KKAy mice (Fig. 6*A* and *B*). In contrast, the expression levels of AdipoR1 and -2 were not affected by rosiglitazone treatment, whereas combined rosiglitazone and Wy-14,643 treatment partially reversed the decrease in these genes (Fig. 6*A* and *B*). We also obtained similar results in BAT (data not shown).

Adiponectin is known to form characteristic multimers (33,34). It was recently reported that the increase in the ratio of the high molecular weight (HMW) form to total adiponectin is correlated with an improvement in insulin sensitivity by TZD treatment (35). Here, we studied whether a PPARα agonist affects the forms of serum adiponectin. As shown in Fig. 6*C*, the ratio of HMW to total adiponectin was decreased in vehicle-treated KKAy mice compared with wild-type KK mice. The restriction of food intake by pair-fed mice partially restored the decrease in the ratio of HMW to total adiponectin in KKAy mice (Fig. 6*C*). Wy-14,643 treatment did not affect the ratio of HMW to total adiponectin, whereas rosiglitazone treatment dramatically increased total adiponectin and the ratio of HMW to total adiponectin (Fig. 6*C*). Treatment with a combination of rosiglitazone and Wy-14,643 showed results similar to rosiglitazone treatment (Fig. 6*C*).

A PPARα agonist directly increased AdipoR expression and suppressed MCP-1 expression. There is a possibility that long-term treatment with Wy-14,643 indirectly increased the gene expression in the WAT of KKAy mice via an improvement in insulin resistance or some other mechanism. Therefore, we examined the effects of short-term treatment with Wy-14,643 on the expression of AdipoR1, AdipoR2, MCP-1, and β3-adrenergic receptor in WAT of KKAy mice. As shown in Fig. 7*A–D*, treatment with

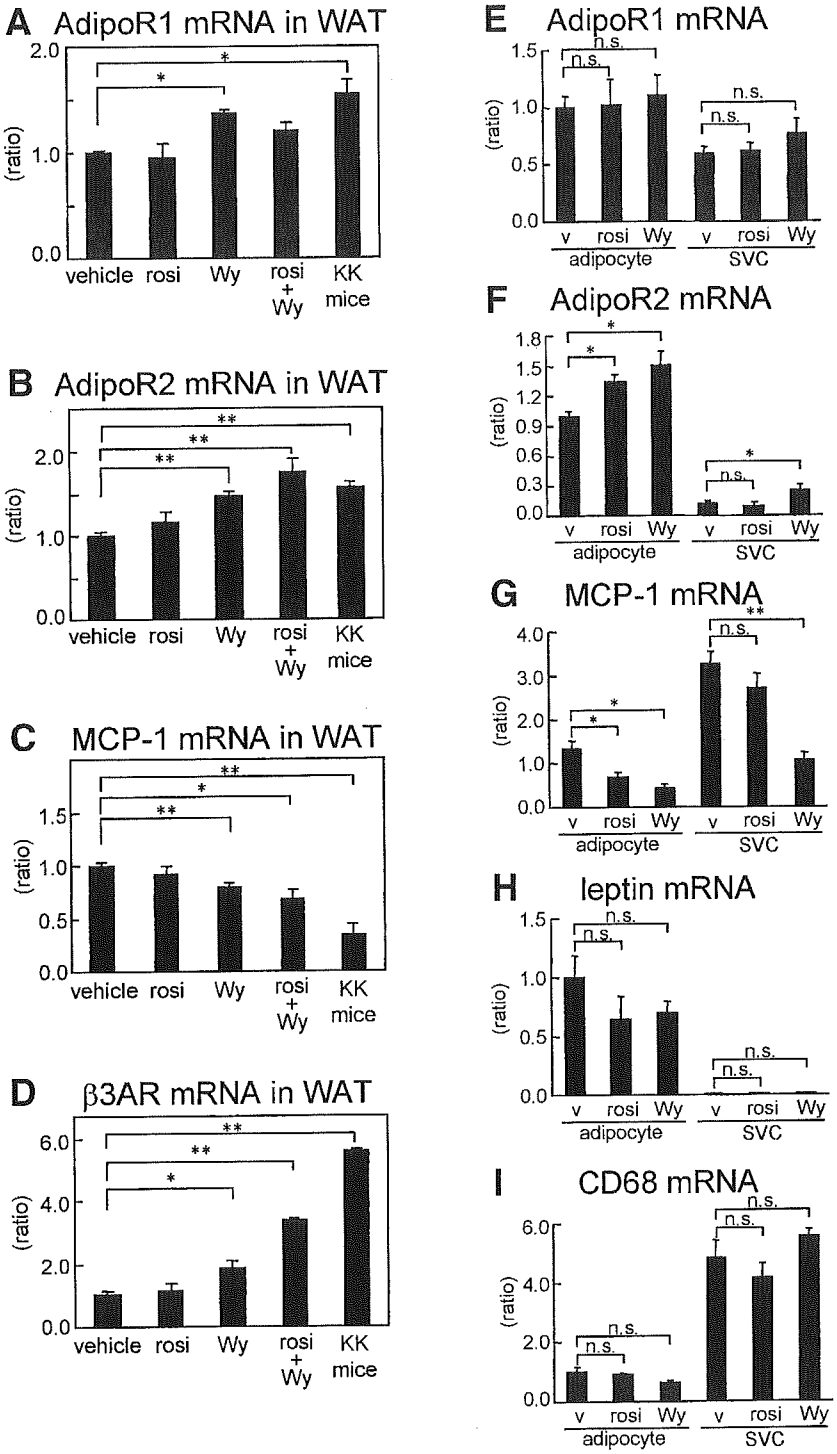

FIG. 7. Effects of rosiglitazone, Wy-14,643, or both rosiglitazone and Wy-14,643 treatment for 3 days on expression of AdipoR1/R2, MCP-1, and β3-adrenergic receptor in WAT of KKAy mice and action of Wy-14,643 on expression of AdipoR1/R2 and MCP-1 in adipocyte (adi) and stromal-vascular cell subfractions of WAT. Panels show amounts of mRNAs of AdipoR1 (*A*), AdipoR2 (*B*), MCP-1 (*C*), and β3-adrenergic receptor (β3AR) (*D*) in epididymal WAT of male KKAy mice treated with 0.01% rosiglitazone (rosi), 0.05% Wy-14,643 (Wy), or both 0.01% rosiglitazone and 0.05% Wy-14,643 (rosi+Wy) as a food admixture for 3 days while on the high-fat diet. Age-matched wild-type KK mice given normal chow were used as normal controls. Amounts of mRNAs of AdipoR1 (*E*), AdipoR2 (*F*), and MCP-1 (*G*) from adipocyte and stromal-vascular cell subfractions of WAT of KKAy mice treated with 0.01% rosiglitazone or 0.05% Wy-14,643 for 3 days while on the high-fat diet. Amounts of the mRNAs of molecules indicated above were quantified by a real-time PCR method as described in RESEARCH DESIGN AND METHODS. The relative amount of each transcript was normalized to the amount of β-actin transcript in the same cDNA. The results are expressed as the ratio of the value of vehicle (v). Each bar represents the means ± SE (n = 3). *P < 0.05; **P < 0.01. n.s., not significant; SVC, stromal-vascular cell.

Wy-14,643 for 3 days increased the expression of AdipoR1, AdipoR2, and β3-adrenergic receptor and decreased the expression of MCP-1 in WAT of KKAy mice. We obtained similar results with the combined treatment of rosiglitazone and Wy-14,643 (Fig. 7*A–D*).

WAT of obese diabetic mice is thought to consist of adipocytes and stromal vascular cells, such as infiltrated macrophages (13,14). We next tried to determine in which cell type the observed effects occur by analyzing the adipocyte and stromal-vascular cell subfractions of adipose tissue separately. As shown in Fig. 7*F* and *G*, treatment with Wy-14,643 for 3 days increased the expression of AdipoR2 and decreased the expression of MCP-1 in both adipocytes and stromal-vascular cells, whereas rosiglitazone treatment showed similar effects only in adipocytes. The expression pattern of leptin and CD68, markers of adipocytes and stromal-vascular cells, respectively, confirmed that the fractionation was performed properly (Fig. 7*H* and *I*).

We next examined the expression of PPARα in the adipose tissue fractions, cultured 3T3-L1 adipocytes, and peritoneal macrophages to determine whether the effects of Wy-14,643 on adipocytes and macrophages were mediated by PPARα. PPARα expression was observed in both the adipose fraction and 3T3-L1 adipocytes as well as in skeletal muscle of KKAy mice (Fig. 8*A*), whereas PPARα