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Angiotensin II infusion decreases plasma adiponectin level via its type 1 receptor in rats: an implication for hypertension-related insulin resistance

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Abstract

We explored the mechanisms underlying the close association between hypertension and insulin resistance by measuring the changes in the plasma levels of adiponectin, a novel insulin sensitizer secreted by adipose tissue, in rats infused with angiotensin II (AII). Angiotensin II (100 ng/kg per minute) was subcutaneously infused with osmotic minipumps for 2 weeks in rats fed with either standard chow or a high-fructose diet. Insulin sensitivity index (SI) was assessed by the minimal model of Bergman [*Diabetes* 1989;38:1512-27]. Angiotensin II infusion significantly increased blood pressure and decreased SI. Angiotensin II decreased plasma adiponectin levels from 3.7 to 2.9 μg/mL ($P < .01$) without affecting the expression of adiponectin messenger RNA in adipose tissue. Angiotensin II infusion did not affect plasma leptin and tumor necrosis factor α levels. An AII type 1 receptor blocker, olmesartan, restored the low adiponectinemia induced by the AII infusion (50 ng/kg per minute). Plasma adiponectin levels were significantly lower in fructose-fed rats (2.3 μg/mL) than in chow-fed rats. Angiotensin II induced no further decrease of adiponectin, whereas olmesartan increased adiponectin remarkably both with and without AII infusion. The AII type 2 receptor blocker PD123319 left the AII-induced hypoadiponectinemia unchanged in both chow- and fructose-fed rats. The AII type 2 receptor agonist CGP42112A also left the adiponectin unchanged. Plasma adiponectin levels were substantially correlated with SI ($r = 0.61$, $P < .0001$). These results suggest that AII suppresses adiponectin production via AII type 1 receptor, resulting in impaired insulin sensitivity.

1. Introduction

Glucose intolerance, hypertension, and dyslipidemia, a triad of established risk factors for cardiovascular diseases, are often clustered in individuals with insulin resistance and its compensatory hyperinsulinemia. Angiotensin II (AII), the major hormone of the renin-angiotensin system, plays an important role in the pathogenesis of hypertension and atherosclerosis [1]. Several lines of evidence have suggested that AII impairs insulin sensitivity [2,3], whereas insulin resistance promotes the development of hypertension by up-regulating the number and activity of AII receptors [4]. Hypertensive subjects and animal models have shown improvements in insulin resistance in response to treatment with angiotensin I converting enzyme inhibitors [5] or AII type 1 receptor (AT1R) blocker [6]. Moreover, the response elicited by the blockers of renin-angiotensin system appeared to be independent of the hypotensive action of the agent. These findings all suggest that AII may have the power to directly inhibit insulin action, but the exact mechanisms for the AII-induced insulin resistance remain largely unknown.

Adiponectin has gained significant attention recently as a mediator of insulin sensitivity [7]. Many clinical and experimental reports have demonstrated decreased circulating levels of this hormone in association with metabolic dysfunctions such as obesity, insulin resistance, and type 2 diabetes mellitus in both humans and animal models [8]. Recent studies have revealed that adiponectin has potential power to prevent the development of insulin resistance [9]. In essential hypertensive patients, Furuhashi et al [10]

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demonstrated that AT1R blocker or angiotensin I converting enzyme inhibitor increased adiponectin concentrations with improvement in insulin sensitivity. Their results prompted us to speculate, firstly, that AII can reduce adiponectin production, and secondly, that the subsequent reduction in circulating adiponectin is involved in the mechanism underlying insulin resistance associated with hypertension. To the best of our knowledge, there has been no study demonstrating the direct effect of AII on plasma adiponectin level in vivo. In this study we sought to characterize the change in plasma adiponectin in response to persistent AII infusion and the association of this change with insulin resistance. We also tried to determine whether it was AT1R or AII type 2 receptor (AT2R) that mediated the action of AII on adiponectin in our experiments. Our group recently found that AT1R blocker, olmesartan medoxomil, improved insulin resistance and dyslipidemia in obese Zucker rats and fructose-fed rats, whereas it exerted no effects on insulin sensitivity or lipid metabolism in lean Zucker rats and rats fed with normal chow. This was unexpected, as the AT1R blocker exerted a similar hypotensive power on both the rats with insulin resistance and those with normal insulin sensitivity [11,12]. This prompted us, in the present study, to additionally examine the effect of AII on plasma levels of adiponectin in insulin-resistant, fructose-fed rats.

2. Materials and methods

Eight-week-old male Wistar rats (Charles River Japan, Tokyo) were divided into 2 groups and fed with 2 diets for a feeding period of 3 weeks. One group was fed with standard rat chow containing 60% vegetable starch, 5% fat, and 24% protein (Oriental Yeast, Tokyo, Japan), whereas the other group was fed with a fructose-rich chow containing 60% fructose, 5% fat, and 20% protein (Oriental Yeast). Both groups were given drinking water with or without 0.01% olmesartan medoxomil (Sankyo, Tokyo, Japan) for the last 14 days of the 3-week period. During the same 14 days, rats from both groups were continuously infused with human AII (Sigma A-9525, St Louis, MO) at 100 ng/kg per minute or saline vehicle alone (controls) by an osmotic minipump (Alzet Model 1007D, Durect Co, Cupertino, CA) implanted subcutaneously [3]. In some chow-fed rats, 10 or 50 ng/kg per minute of AII was infused for 14 days. All rats were kept in individual cages on a rotating 12-hour light-dark cycle with free access to food and water. On the day of the experiment, the animals were fasted from 9:00 AM up to the commencement of the experiment at 2:00 PM. Drinking water remained available. All procedures were approved by the Institutional Animal Care and Use Committee of Showa University according to the Guidelines for the Care and Use of Laboratory Animals.

The infusion rate of AII, 100 ng/kg per minute, is a common dosage used by many other researchers [3,13]. When we measured the plasma AII concentration by radioimmunoassay in rats infused with AII (100 ng/kg per minute) or saline in preliminary studies, we found that the AII level was only 2-fold higher in AII-infused rats than in the saline-infused rats (220 ± 41 vs 120 ± 37 pg/mL) (data not shown). In one study, the plasma AII concentration in patients with renovascular hypertension was reportedly 3-fold higher than that in controls [14], suggesting that the plasma AII levels in our AII-infused rats are within the physiologic range. The renin-angiotensin system is locally stimulated in most of the hypertensive subjects even when their plasma AII level remains within a reference range [1,15]. Thus, AII-infused rats seem to be a suitable animal model for hypertension with increased AII action.

2.1. PD123319 treatment

In some of the rats infused with 100 ng/kg per minute of AII, the AT2R blocker PD123319 (Sigma P-186) was simultaneously infused at 30 mg/kg per day for the last 7 days of the 2-week AII infusion by an osmotic minipump (Alzet Model 2ML1, Durect Co) implanted subcutaneously (n = 6). This dose of PD123319 has been shown to exert an effective AT2R blockade in rats [16]. We scheduled the PD123319 infusion for the last 7 days of the AII infusion because the agent has a very high infusion rate and needs a large size of minipump (2 mL). Furthermore, 1- and 2-week infusions of PD123319 did not bring about significantly effects on lipid and glucose metabolism in our preliminary study (data not shown). In control rats (n = 4), AII was infused together with saline via the same type of minipump.

2.2. CGP42112A treatment

CGP42112A (4296V, Peptide Research Lab, Osaka, Japan), an agonist of AT2R, was infused at 1 μg/kg per minute for 14 days by an osmotic minipump (Alzet Model 2ML2, Durect Co) implanted subcutaneously (n = 4) [17]. In control rats (n = 3), saline was infused via the same type of minipump.

2.3. Blood pressure and heart rate measurement

The systolic blood pressure (SBP), diastolic BP (DBP), and heart rate (HR) were recorded in completely conscious rats using indirect tail-cuff equipment (Natsume Seisakusho, Tokyo, Japan). After prewarming the rats on a 37°C plate for 20 minutes, the BP and HR were each measured 5 times. The mean values were taken as the individual BP and HR of each rat.

2.4. Frequently sampled intravenous glucose tolerance test

The frequently sampled intravenous glucose tolerance test (FSIVGTT) was performed to evaluate insulin sensitivity index (SI) and glucose effectiveness (SG) by the minimal model method of Bergman [18]. As there were no significant differences in glucose and insulin levels between chow-fed rats with and without olmesartan during the intravenous glucose tolerance test in our previous study [12], we did not perform the FSIVGTT in the chow-fed rats with olmesartan treatment. Under an anesthetized state

with pentobarbital, a heparin-filled catheter was placed into a femoral vein 1 hour before the experiment, a bolus injection of glucose solution (50%, at a dose of 0.5 g/kg) was administered at the basal state, and blood samples (0.15 mL for each time point) were taken at −5, 0, 2, 4, 6, 8, 10, 12, 14, 16, 19, 22, 30, 40, 60, 90, and 120 minutes. Plasma was separated by centrifugation at 4°C and stored at −20°C for subsequent measurement of glucose and insulin. SI and SG in the minimal model approach of Bergman [18] represent insulin-dependent and insulin-independent glucose disposal abilities in vivo, respectively. The Marquardt-Levenberg method is used for nonlinear least-square estimation of the parameters. The values at 2 to 6 minutes after the glucose injection are zero-weighted, and the step size for integration is 1 minute. The plasma glucose disappearance rate constant (K_g) is calculated as the slope of the least-square regression line relating the natural logarithm of the glucose concentration between 4 and 16 minutes.

2.5. Gene expression of adiponectin in adipose tissue

We examined adiponectin gene expression in epididymal and subcutaneous adipose tissues of rats infused with AII (n = 6) and vehicle (saline, n = 6). Total RNA was extracted from adipose tissues using RNeasy Lipid Tissue Mini Kit (Qiagen). RNA quality and quantity were assessed using automated capillary gel electrophoresis on a Bioanalyzer 2100 with RNA Nano LabChips (Agilent Technology, Tokyo, Japan). Reverse transcription was carried out using 800 ng of total RNA with ReverTra Dash reverse transcriptase–polymerase chain reaction (RT-PCR) kit (Toyobo) and Oligo(dT)20 primer. Polymerase chain reaction was done for 25 cycles (98°C for 10 seconds, 60°C for 2 seconds, 74°C for 30 seconds, and a final elongation step of 7 minutes at 72°C) for adiponectin and β-actin. The following primers were used: adiponectin forward, 5′-GCT CTG GTC CCT CCA CCC-3′; adiponectin reverse, 5′-GCC GTC ATA ATG ATT CTG TTG G-3′(GenBank accession number AY033885); β-actin forward, 5′-ACT GGC ATT GTG ATG GAC TC-3′; β-actin reverse, 5′-GTG GTG GTG AAG CTG TAG CC-3′ (GenBank accession number NW031144). The products of RT-PCR were analyzed with DNA 7500 LabChip Kit (Agilent Technology). All chips were prepared according to the manufacturer's instructions.

2.6. Biochemical assays

Plasma adiponectin (Ootsuka, Osaka, Japan), leptin (Morinaga, Yokohama, Japan), insulin (Morinaga), and tumor necrosis factor α (TNF-α) (Pierce, Boston, MA), respectively, were measured by enzyme-linked immunosorbent assay kits for rats. Glucose was measured in duplicate spectrophotometers with standard commercial kits (Wako Pure Chemical Industries, Osaka, Japan).

2.7. Statistical analysis

Results are expressed as mean ± SD. One-way analysis of variance and the independent *t* test were used to evaluate differences of means between different groups. Total trends of parameters measured in the time-course study for AII and saline infusion groups were compared by a general linear model of repeated measures. Correlation between 2 parameters was analyzed using Pearson simple correlation analysis. Statistical significance was accepted at $P < .05$.

3. Results

3.1. General profiles and plasma adiponectin levels in chow-fed rats infused with different dosages of AII or vehicle (saline) with or without olmesartan treatment

As shown in Table 1, the AII infusions at dosages of both 50 and 100 ng/kg per minute significantly and

Table 1
General profiles and plasma adiponectin levels in chow-fed rats with and without AII infusion, as well as treatment with olmesartan

Olmesartan	Vehicle		AII (10 ng/[kg · min])	A II (50 ng/[kg · min])		A II (100 ng/[kg · min])	
	(−)	(+)		(−)	(+)	(−)	(+)
n	15	11	8	12	5	18	17
Body weight (g)	401 ± 17	452 ± 19	394 ± 16	402 ± 9	402 ± 5	405 ± 10	413 ± 24
Food intake (g/d)	33.5 ± 5.1	30.8 ± 3.6	34.8 ± 10.0	33.9 ± 6.9	36.3 ± 7.5	31.3 ± 5.0	30.9 ± 9.4
Water intake (mL/d)	46.3 ± 7.2	44.0 ± 3.8	44.6 ± 12.3	46.3 ± 8.1	49.2 ± 3.6	48.6 ± 10.3	47.3 ± 6.5
SBP (mm Hg)	110 ± 5	100 ± 13	135 ± 8**	142 ± 6**	118 ± 3‡	151 ± 18**	119 ± 12#
DBP (mm Hg)	79 ± 6	70 ± 9*	93 ± 8**	107 ± 5**	87 ± 6‡	106 ± 14**	82 ± 10#
HR (beats/min)	371 ± 36	355 ± 35	380 ± 23	382 ± 45	375 ± 16	370 ± 29	366 ± 24
WAT (g)	3.2 ± 0.7	NA	3.4 ± 0.1	3.1 ± 0.7	3.4 ± 1.1	3.1 ± 0.7	3.2 ± 0.5
Glucose (mg/dL)	159.2 ± 12.2	147.2 ± 25.1	162.9 ± 13.4	170.3 ± 17.4	170.2 ± 16.6	161.2 ± 14.8	166.5 ± 12.0
Insulin (μU/L)	28.8 ± 11.5	32.9 ± 11.3	58.6 ± 14.7**	59.7 ± 12.6**	51.1 ± 12.9**	59.5 ± 10.9**	60.3 ± 11.9**
Adiponectin (μg/mL)	3.69 ± 0.69	3.89 ± 0.47	3.70 ± 0.40	2.98 ± 0.34**,†	4.26 ± 0.55‡	2.94 ± 0.27**,†	3.14 ± 0.92*

Data were expressed as mean ± SD. These results were collected 14 days after the commencement of the AII or saline infusions. WAT indicates weight of epididymal fat pads.

* $P < .05$ vs vehicle without olmesartan group.

** $P < .01$ vs vehicle without olmesartan group.

† $P < .01$ vs AII (10 ng/kg per minute) without olmesartan group.

‡ $P < .01$ vs AII (50 ng/kg per minute) without olmesartan group.

\# $P < .01$ vs AII (100 ng/kg per minute) without olmesartan group.

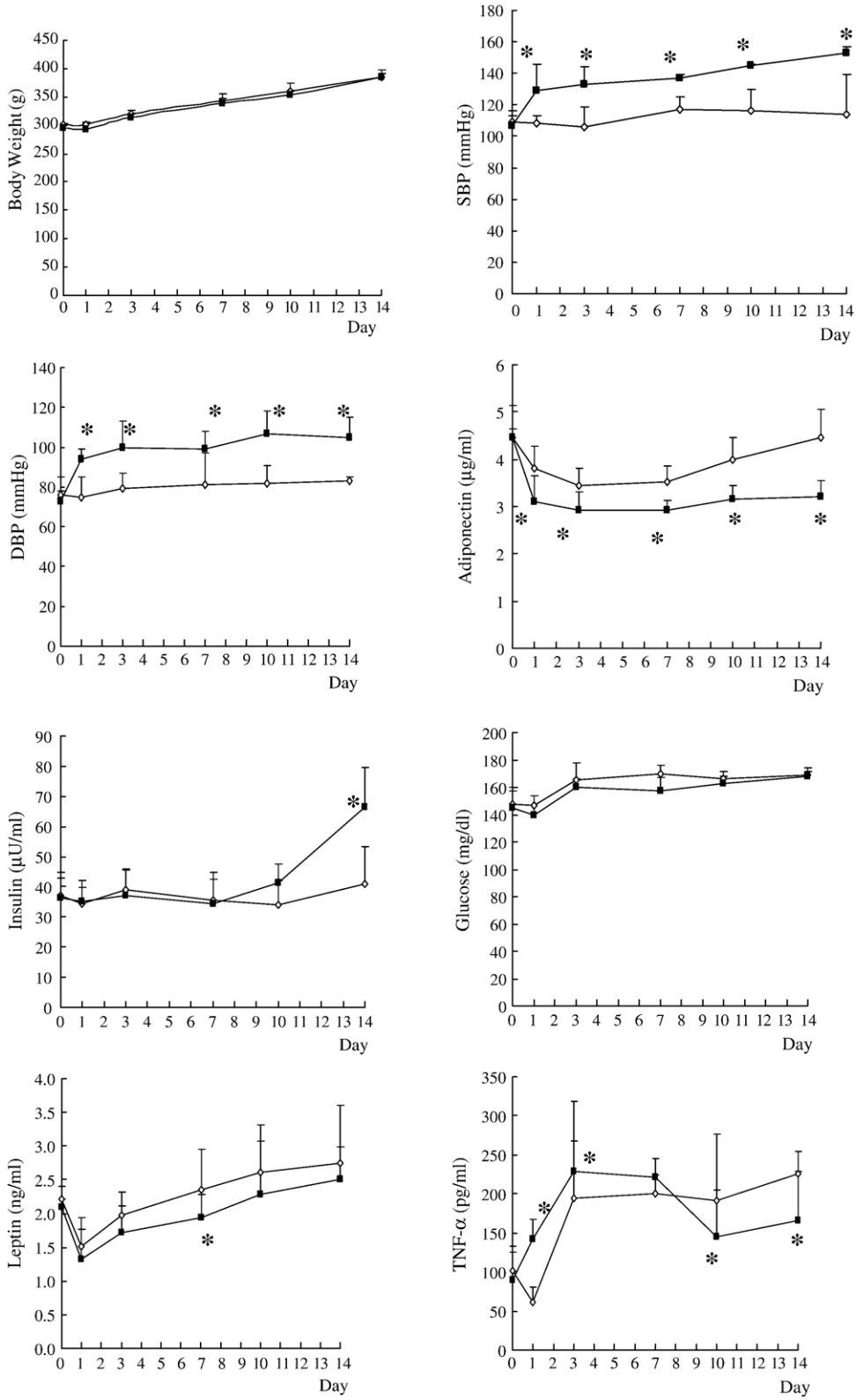

Fig. 1. Time-course investigation of various parameters after saline (◇) and AII (■) (100 ng/kg per minute) infusion for 2 weeks in chow-fed rats. Data were expressed as mean ± SD. General line model analysis of repeated measure showed that body weight, and plasma concentrations of glucose, leptin, and TNF-α were comparable between 2 groups, whereas the curve trends of SBP, DBP, and plasma concentrations of adiponectin and insulin between 2 groups were quite different. *$P < .01$, significant vs saline infusion group at a corresponding time point.

Table 2
The effects of AT2R blocker (PD123319) or agonist (CGP42112A) on general profile and plasma adiponectin level in chow-fed rats

	A			B		
	AII	AII + PD	*P*	Saline	CGP	*P*
n	4	6	–	3	4	–
Body weight (g)	389 ± 10	399 ± 21	NS	390 ± 10	399 ± 30	NS
Food intake (g/d)	34.9 ± 5.1	36.4 ± 10.3	NS	33.2 ± 11.2	35.8 ± 10.8	NS
Water intake (mL/d)	45.7 ± 10.2	45.9 ± 3.9	NS	47.7 ± 5.7	50.3 ± 6.8	NS
SBP (mm Hg)	138 ± 9	142 ± 10	NS	119 ± 7	121 ± 13	NS
DBP (mm Hg)	94 ± 9	92 ± 8	NS	85 ± 8	76 ± 20	NS
HR (beats/min)	378 ± 13	363 ± 19	NS	395 ± 37	384 ± 36	NS
WAT (g)	3.0 ± 0.6	2.9 ± 0.6	NS	3.2 ± 0.3	3.2 ± 1.0	NS
Glucose (mg/dL)	176.1 ± 11.8	181.8 ± 18.0	NS	173.9 ± 7.8	172.5 ± 6.3	NS
Insulin (μU/mL)	68.7 ± 17.8	54.9 ± 16.1	NS	33.1 ± 1.8	31.9 ± 5.3	NS
Adiponectin (μg/mL)	2.71 ± 0.54	2.50 ± 0.66	NS	3.09 ± 0.28	3.37 ± 0.30	NS

Data were expressed as mean ± SD. *P* values were according to independent *t* test. NS indicates no significance.

dose-dependently elevated the SBP and DBP without changing the HR, and olmesartan treatment completely rectified the AII-induced hypertension. Olmesartan alone slightly decreased the DBP in the saline-infused rats, but it did not affect the SBP. Neither AII nor olmesartan exerted any affects on food intake, water intake, body weight, or weight of the epididymal fat pads. Angiotensin II infusions at all infusion rates remarkably increased the plasma insulin without affecting the plasma glucose level. Olmesartan treatment did not suppress the hyperinsulinemia induced by the AII infusion at the dose of 100 ng/kg per minute, but it slightly decreased the plasma insulin level in the animals infused at the dose of 50 ng/kg per minute. Olmesartan alone had no effect on the plasma levels of glucose and insulin (Table 1). The 100 and 50 ng/kg per minute AII infusions both decreased the plasma adiponectin level by

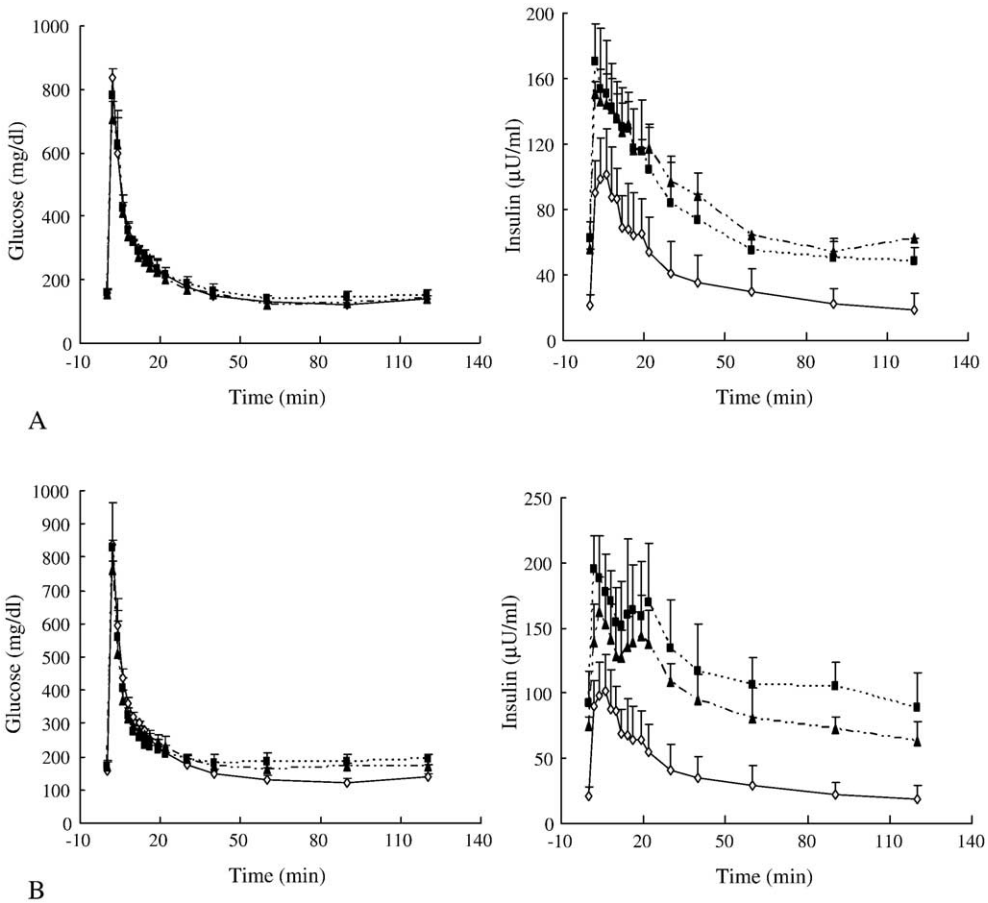

Fig. 2. Glucose and insulin levels during FSIVGTT in chow-fed rats with different AII infusion rate: 100 ng/kg per minute (A) and 50 ng/kg per minute (B) for 2 weeks, with and without olmesartan treatment. The experiment was performed 14 days after the commencement of AII or saline infusions. Data are expressed as mean ± SD. ◇ indicates saline-infused group as normal control; ■, AII-infused group; ▲, AII infusion with olmesartan treatment.

Table 3
Parameters calculated by the minimal model of Bergman for chow-fed rats in FSIVGTT

Olmesartan	Vehicle		AII (100 ng/[kg · min])		AII (50 ng/[kg · min])	
	(−)	(+)	(−)	(+)	(−)	(+)
n	4	6	6	3	4	5
AUCG (mg/[dL · min]) ($\times 10^4$)	2.08 ± 0.13	2.00 ± 0.26	2.30 ± 0.15	2.12 ± 0.11	2.59 ± 0.16	2.44 ± 0.09
AUCI (μU/[mL · min]) ($\times 10^4$)	0.44 ± 0.14	0.45 ± 0.14	0.89 ± 0.13**	0.89 ± 0.03	1.44 ± 0.25**	1.11 ± 0.17*
K_g (%/min)	4.19 ± 0.61	4.80 ± 0.66	4.01 ± 0.89	5.03 ± 1.05	4.84 ± 0.57	4.03 ± 0.88
SI (μU/[mL · min]) ($\times 10^{-4}$)	6.65 ± 2.67	7.29 ± 2.69	2.07 ± 0.59**	2.89 ± 0.37	2.24 ± 0.83**	3.61 ± 0.54*
SG (per min) ($\times 10^{-2}$)	3.81 ± 0.86	4.50 ± 1.19	4.40 ± 0.98	4.40 ± 0.14	4.55 ± 0.84	3.75 ± 0.65

Data were expressed as mean ± SD. AUCG, AUCI, SI, SG, and K_g are parameters assessed by the minimal model of Bergman [18].
* $P < .05$ vs AII (50 ng/kg per minute) without olmesartan group.
** $P < .01$ vs vehicle without olmesartan group.

27%, whereas the low-dose infusion (10 ng/kg per minute) left the plasma adiponectin unchanged (Table 1). Olmesartan did not rectify the hypoadiponectinemia induced by the 100 ng/kg per minute AII infusion, whereas it completely rectified that induced by the 50 ng/kg per minute infusion. The administration of olmesartan alone left the plasma adiponectin unchanged in the saline-infused rats (Table 1).

3.2. General profiles and plasma levels of other adipocytokines during AII (100 ng/kg per minute) infusion for 2 weeks

Fig. 1 depicts changes in various parameters during infusions with AII and saline. Angiotensin II infusion significantly elevated the SBP and DBP within 1 day and maintained the high levels up to 14 days without affecting body weight. Angiotensin II decreased the plasma adiponectin within 1 day and kept it low throughout the infusion. The plasma glucose showed no change whatsoever during the infusion period. The plasma insulin levels remained unchanged up to the 10th day of the infusion, then rose to a significantly elevated level by the 14th day of the infusion. Plasma TNF-α and leptin levels varied significantly after the commencement of the infusion, but there were no significant differences in the overall trends between the AII and saline infusion groups.

3.3. The effects of PD123319 and CGP42112A on plasma adiponectin levels

We examined the role of AT2R in AII-induced hypoadiponectinemia. The simultaneous infusion of PD123319, a specific antagonist of AT2R, did not affect the general profiles and plasma levels of glucose, insulin, and adiponectin in the AII-infused chow-fed rats (Table 2A). We also examined the effect of CGP42112A, a specific agonist of AT2R, in chow-fed rats (Table 2B). CGP42112A did not affect the general profiles and plasma levels of insulin and adiponectin.

3.4. Plasma glucose and insulin levels in FSIVGTT

Fig. 2 depicts the changes in the FSIVGTT results on plasma glucose and insulin levels in the saline-infused rats and rats infused 100 and 50 ng/kg per minute of AII with or without olmesartan treatment. Table 3 indicates parameters obtained by assessment of the minimal model of Bergman. The AII-infused rats showed marked hypersecretion of insulin in the FSIVGTT without any changes of plasma

Table 4
General profiles in fructose-fed rats with and without AII infusion, as well as treatment with olmesartan

Olmesartan	Chow-fed	Fructose-fed		Fructose-fed with AII (100 ng/[kg · min])	
		(−)	(+)	(−)	(+)
n	15	10	11	12	15
Body weight (g)	401 ± 17	364 ± 22	371 ± 22	377 ± 20	368 ± 20
Food intake (g/d)	33.5 ± 5.1	17.1 ± 4.5**	17.2 ± 2.6**	16.7 ± 7.8**	17.0 ± 6.9**
Water intake (mL/d)	46.3 ± 7.2	44.4 ± 3.6	50.0 ± 3.5	46.7 ± 10.1	49.3 ± 6.1
SBP (mm Hg)	110 ± 5	115 ± 4	90 ± 18††	147 ± 7**	92 ± 16‡
DBP (mm Hg)	79 ± 6	87 ± 4*	63 ± 18††	103 ± 6**	64 ± 11‡
HR (beats/min)	371 ± 36	364 ± 27	387 ± 40	371 ± 23	387 ± 12
Glucose (mg/dL)	159.2 ± 12.2	159.9 ± 13.2	153.7 ± 23.9	215.9 ± 26.7**,††	164.7 ± 11.2‡
Insulin (μU/mL)	28.8 ± 11.5	92.7 ± 21.2**	92.0 ± 23.0**	131.4 ± 25.8**,††	78.3 ± 9.4‡
Adiponectin (μg/mL)	3.69 ± 0.69	2.33 ± 0.47**	4.26 ± 0.57††	2.26 ± 0.50**	3.52 ± 1.00††‡

Data were expressed as mean ± SD.
* $P < .05$ vs chow-fed rats.
** $P < .01$ vs chow-fed rats.
† $P < .05$ vs fructose-fed rats without olmesartan.
†† $P < .01$ vs fructose-fed rats without olmesartan.
‡ $P < .01$ vs AII-infused rats without olmesartan.

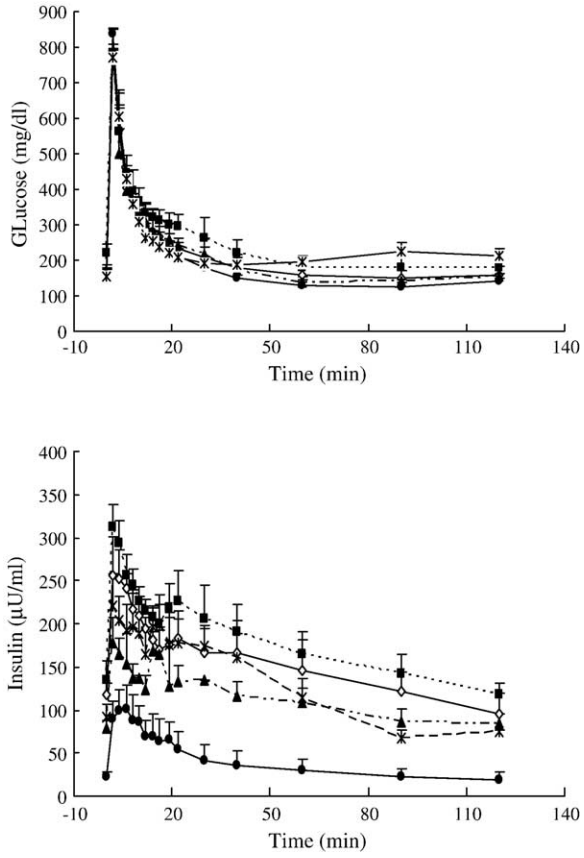

Fig. 3. Glucose and insulin levels during FSIVGTT in fructose-fed rats. The experiment was performed 14 days after commencement of AII or saline infusions. Data are expressed as mean ± SD. ● indicates chow-fed rats group; ◇, saline-infused group; *, fructose-fed rats with olmesartan treatment; ■, AII-infused group; ▲, AII infusion with olmesartan treatment.

glucose levels. Thus, the area under the curve of insulin (AUCI) was doubled without affecting the area under the curve of glucose (AUCG). Insulin sensitivity index was markedly decreased in the AII-infused rats compared with that in the saline-infused rats. None of the AII doses influenced the insulin-independent glucose disposal (SG) or glucose disappearance constant (K_g). The olmesartan treatment failed to ameliorate the hypersecretion of insulin in the AII (100 ng/kg per minute)–infused rats (Fig. 2A), whereas it significantly suppressed the hypersecretion of insulin induced by the 50 ng/kg per minute AII infusion (Fig. 2B). The olmesartan treatment significantly increased SI in the AII (50 ng/kg per minute)–infused group (Table 3).

3.5. General profiles and plasma adiponectin levels in fructose-fed rats infused with AII (100 ng/kg per minute) or vehicle (saline) with or without olmesartan treatment

Fructose-fed rats had a marginally increased BP compared with chow-fed rats. As with the chow-fed rats, the fructose-fed animals treated with olmesartan exhibited a significantly decreased SBP and DBP. Angiotensin II infusion for 14 days significantly increased BP, and olmesartan treatment completely abolished the AII-induced hypertension. Indeed, BP was comparable in the animals treated with olmesartan alone and the animals treated with olmesartan plus AII (Table 4). The saline-, AII infusion–, and olmesartan-treated groups fed with the high-fructose diet exhibited no reductions in body weight, food intake, or water intake. The plasma insulin level was 3-fold higher in the fructose-fed rats than in the chow-fed rats, whereas the plasma glucose levels were comparable between these 2 groups. Olmesartan did not affect the plasma insulin and glucose in the fructose-fed rats (Table 4). Angiotensin II infusion further increased plasma glucose and insulin levels in the fructose-fed rats, whereas olmesartan significantly ameliorated the hyperglycemia and hyperinsulinemia induced by the AII infusion (Table 4). The plasma adiponectin was significantly lower in the fructose-fed rats than in the chow-fed rats. The olmesartan treatment completely normalized the hypoadiponectinemia induced by the fructose feeding. The fructose-fed rats infused with AII exhibited no further decrease of plasma adiponectin, whereas the olmesartan treatment significantly increased adiponectin up to the normal level (Table 4).

We also examined the effect of AT2R on the plasma adiponectin level by simultaneously infusing PD123319 in the fructose-fed rats. The PD123319-induced change in the plasma adiponectin level did not significantly differ

Table 5
Parameters calculated by Bergman minimal model for fructose-fed rats in FSIVGTT

Olmesartan	Chow-fed	Fructose-fed		Fructose-fed with AII (100 ng/[kg · min])	
		(−)	(+)	(−)	(+)
n	4	4	6	6	3
AUCG (mg/[dL · min]) ($\times 10^4$)	2.08 ± 0.13	2.42 ± 0.25	2.28 ± 0.33	2.83 ± 0.33**,†	2.32 ± 0.25
AUCI (μU/[mL · min]) ($\times 10^4$)	0.44 ± 0.14	1.82 ± 0.27**	1.40 ± 0.33**,††	2.09 ± 0.24**,††	1.23 ± 0.07**,‡
K_g (%/min)	4.19 ± 0.61	3.44 ± 0.37*	5.00 ± 0.69††	3.01 ± 0.27**	3.96 ± 0.19
SI (μU/[mL · min]) ($\times 10^{-4}$)	6.65 ± 2.67	1.12 ± 0.53**	1.92 ± 0.34**,††	0.13 ± 0.03**,††	1.92 ± 0.99**,‡
SG (per min) ($\times 10^{-2}$)	3.81 ± 0.86	4.36 ± 0.79	4.46 ± 0.70	6.80 ± 0.93**,†	4.26 ± 0.48

Data were expressed as mean ± SD.
* $P < .05$ vs chow-fed rats.
** $P < .01$ vs chow-fed rats.
† $P < .05$ vs fructose-fed rats without olmesartan.
†† $P < .01$ vs fructose-fed rats without olmesartan.
‡ $P < .01$ vs AII-infused rats without olmesartan.

between the AII-infused fructose-fed rats (n = 8) and their saline-infused counterparts (n = 6) (2.26 ± 0.50 vs 2.31 ± 0.30 μg/mL).

3.6. Plasma glucose and insulin levels in FSIVGTT in fructose-fed rats

Fig. 3 shows the FSIVGTT results on changes in the plasma glucose and insulin levels in fructose-fed rats infused with AII (100 ng/kg per minute) or saline with or without olmesartan treatment. The plasma glucose and insulin profiles in chow-fed rats are also shown as controls. Plasma insulin responses were markedly higher in the fructose-fed rats than in the chow-fed rats. Angiotensin II infusion augmented the hyperinsulin responses in the fructose-fed rats with significantly increased glucose responses. The olmesartan treatment significantly suppressed the hyperresponse of insulin and ameliorated glucose intolerance induced by the AII infusion in the fructose-fed rats. Table 5 indicates parameters obtained by assessment of the minimal model of Bergman. The AUCI was 4-fold higher in the fructose-fed rats than in the chow-fed rats, whereas the AUCG was comparable between the 2 groups. Thus, SI was decreased 6-fold in the fructose-fed rats compared with that in the chow-fed rats. The olmesartan treatment significantly decreased AUCI and increased SI without affecting the AUCG and SG. The AII infusion further increased the AUCI and significantly increased the AUCG in the fructose-fed rats, resulting in a substantial decrease of SI. The AII infusion significantly increased SG. The olmesartan treatment rectified the additive effects of AII over that induced by fructose feeding (Table 5).

3.7. Relation between adiponectin and insulin resistance

Fig. 4 depicts the relationship between the plasma adiponectin and SI determined by the minimal model of Bergman in both chow- and fructose-fed rats examined by the FSIVGTT. There was an excellent correlation between the plasma adiponectin and SI ($r = 0.61$, $P < .0001$, n = 40) in the total population of animals that underwent the test.

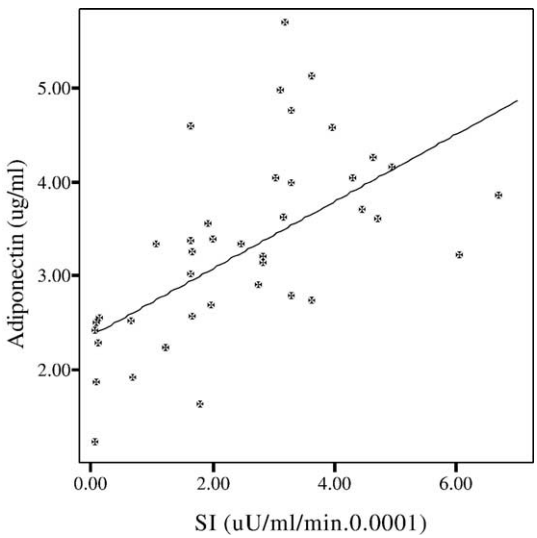

Fig. 4. Correlation between plasma adiponectin level and SI in rats fed with normal chow and fructose-rich diet. Pearson correlation analysis showed that $r = 0.605$ ($P < .0001$).

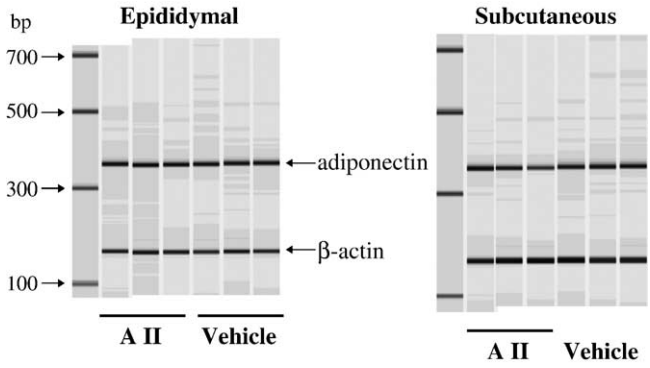

Fig. 5. Adiponectin gene expression in the epididymal and subcutaneous adipose tissues of AII- and vehicle (saline)–infused rats.

3.8. The effect of AII infusion on adiponectin gene expression in the epididymal and subcutaneous adipose tissues

Fig. 5 shows the representative picture of adiponectin gene expression in the epididymal and subcutaneous adipose tissues of AII (100 ng/kg per minute)– and vehicle-infused rats. The AII infusion did not affect the expression of adiponectin messenger RNA (mRNA) in both visceral and subcutaneous adipose tissues. The adiponectin mRNA–β-actin mRNA ratios in epididymal fat tissue were 1.15 ± 0.11 and 1.14 ± 0.18 in saline-infused rats (n = 6) and AII-infused rats (n = 6), respectively. The ratios in subcutaneous fat tissue were 1.08 ± 0.12 and 0.99 ± 0.14 in saline-infused rats (n = 6) and AII-infused rats (n = 6), respectively.

4. Discussion

Several lines of evidence point to an association between AII and insulin resistance [2,3]. The reported mechanisms include AII-induced hemodynamic changes in tissue glucose and insulin utilization [19], impairment of intracellular insulin signaling, and increased oxidative stress [3]. The adipocytokines TNF-α, resistin, and plasminogen activator inhibitor 1 (PAI-1) have recently been demonstrated to play critical roles in the process of insulin resistance [20]. In vitro studies on different types of adipocyte suggest that AII is a positive regulator of PAI-1 synthesis [21]. Skurk et al [22] demonstrate that AII and its metabolites promote PAI-1 production and release from human adipocytes, processes which might contribute to insulin action in obesity. All these findings suggest that AII may induce insulin resistance via its regulative effects on active adipokines.

Adiponectin, a novel adipose tissue-specific cytokine that circulates in plasma at high levels [23], has been demonstrated to increase insulin sensitivity by increasing

tissue fat oxidation and decreasing the influx of free fatty acid into the liver [23], reducing the intracellular triglyceride content in the liver and muscle [24], suppressing the hepatic glucose output, and increasing the muscular glucose utilization [23]. In this study we sought to determine whether AII reduces plasma adiponectin level and thereby leads to a state of hypoadiponectinemia that facilitates the development of insulin resistance. The examination by the minimal model of Bergman in our study indicated that long-term AII infusion impairs insulin-mediated glucose disposal in normal rats. However, insulin-independent glucose disposal (SG) shows no change in response to AII infusion. As such, we speculate that the AII-induced insulin resistance may be more attributable to the direct impairment of intracellular insulin action [25] than to the hemodynamic changes that diminish the glucose diffusion from the vessels and capillaries to the insulin-independent tissues [19]. Angiotensin II infusion (50 and 100 ng/kg per minute) significantly reduced the plasma adiponectin. The hypoadiponectinemia developed soon after the commencement of AII infusion, whereas insulin resistance did not appear until the 14th day of the infusion. We found a positive correlation between plasma adiponectin level and SI, supporting the *impossible* contention that the reduced level of circulating adiponectin contributes to the development of AII-induced insulin resistance. However, the lower infusion rate of AII (10 ng/kg per minute) increased the plasma insulin level without affecting the plasma adiponectin, suggesting that insulin resistance/hyperinsulinemia induced by AII infusion may not always be associated with hypoadiponectinemia. In vivo, AII may regulate adiponectin secretion in an autocrine/paracrine manner like its action on other adipokines [22], the low infusion rate might not elicit enough pathologic concentration in the local adipose tissues. As shown in previous investigations [3,25,26], AII may directly impair the intracellular insulin signaling pathway in addition to influencing adiponectin.

How does AII reduce circulating adiponectin? Earlier reports have suggested that plasma adiponectin levels were negatively correlated to changes in body weight and the weight of the white adipose tissue [27]. Long-term AII infusion, however, did not affect body weight or the mass of epididymal fat tissues in either chow-fed or fructose-fed rats. Leptin, another adipose tissue–specific protein that circulates in the plasma, usually reflects adiposity in the whole body [28]. Kim et al [29] demonstrated an AII-induced increase in the gene expression and secretion of leptin by cultured adipocytes. In contrast to their in vitro observation, the AII infusion did not affect the plasma leptin level in the rats in our study. Cassis et al [30] also failed to find an increase of plasma leptin level after AII infusion. To explain their result, they speculated that the AII infusion may stimulate a sympathetic nerve activity that cancels the potential stimulating power of AII on leptin production. In any case, the AII-induced reduction in circulating adiponectin does not appear to be solely due to increased adiposity.

Tumor necrosis factor α may be an important modulator for adipose adiponectin secretion. Ouchi et al [31] demonstrated that TNF-α down-regulates adiponectin gene expression. Tumor necrosis factor α impairs the signal transduction of insulin in skeletal muscles, resulting in increased insulin resistance [31]. Togashi et al [32] reported an increase in the TNF-α content in skeletal muscles in a model of hypertensive insulin-resistant rat fed with a high-fructose diet. Judging from their results, the TNF-α molecule may mediate the power of AII to suppress adiponectin synthesis. Although we did not observe the elevation of plasma TNF-α in the AII-infused rats, it remains the possibility that AII stimulates TNF-α production in adipose tissues, which might interfere with adiponectin production.

According to the hypothesis proposed by Sharma et al [33], AII markedly inhibits differentiation of human adipocytes and thereby may impair the recruitment and differentiation of preadipocytes, resulting in increases in the number of large adipocytes and decreases in the number of small adipocytes. The impaired adipogenesis may decrease adiponectin secretion by decreasing its net capacity for production [34]. We did not find that AII infusion significantly suppresses adiponectin gene expression in both visceral and subcutaneous adipose tissues by the semiquantitative RT-PCR analysis, although AII significantly decreased plasma adiponectin level. Several previous studies also failed to find a correlation between plasma adiponectin and its mRNA expression in adipose tissue [20]. Fasshauer et al [35] found that AII left the gene expression of adiponectin in 3T3-L1 adipocytes unchanged. These observations suggest that posttranscriptional processes are more important determinants of the plasma level of adiponectin. There is a possibility that AII inhibits insulin-mediated adiponectin exocytosis by suppressing phosphoinositide 3-kinase activity in adipocytes [36]. Nevertheless, Hattori et al [37] recently reported that AII-induced oxidative stress and endothelial dysfunction are accompanied by a decrease in adiponectin gene expression in either rats' adipose tissue or in cultured 3T3-L1 adipocytes, and antioxidants were observed to prevent the actions of AII; they concluded that adiponectin gene expression is negatively modulated by oxidative stress. Gathering these in all, further experiments will be *essential* to examine the effect of AII on adiponectin production and secretion in adipose tissues.

Insulin resistance and hyperinsulinemia have been confirmed as negative modulators of adiponectin secretion [38]. On the other hand, our present study showed that hypoadiponectinemia far preceded the AII-induced development of insulin resistance/hyperinsulinemia, suggesting that the reduced circulating adiponectin stemmed from something other than *secondary to* the insulin resistance/hyperinsulinemia.

In the present study, olmesartan failed to restore the AII (100 ng/kg per minute)–induced hypoadiponectinemia. We

speculated that the high infusion rate of AII (100 ng/kg per minute) relative to the olmesartan dosage may have prevented the latter from attenuating the action of the former. In a second attempt, therefore, we repeated the same experiment with a low AII infusion rate of 50 ng/kg per minute. As expected, olmesartan completely rectified the AII-induced hypoadiponectinemia. Similarly, olmesartan failed to improve insulin resistance induced by the high rate of AII infusion (100 ng/kg per minute), although it significantly improved insulin sensitivity when the rate of AII infusion was low (50 ng/kg per minute). Olmesartan completely suppressed the increase in the BP induced by the AII infusions at both infusion rates, whereas it only improved the low adiponectin and insulin resistance in the animals infused with the low dose of AII. These results suggest that the suppressive power of AII on adiponectin and insulin sensitivity is stronger than its hypertensive action.

Another novel finding of this study was the significant decrease in the plasma adiponectin in the fructose-fed rats, a representative animal model of insulin resistance. It may be that the hypoadiponectinemia is involved in the mechanisms for insulin resistance in these animals. Angiotensin II brought about no further decrease in the adiponectin level in fructose-fed rats, suggesting that fructose feeding and AII infusion might reduce the plasma adiponectin level via a common mechanism. Olmesartan treatment completely rectified the hypoadiponectinemia caused by the fructose feeding in rats with and without AII infusion, suggesting that both fructose feeding and AII infusion suppress adiponectin production via AT1R. Certain ARBs, such as irbesartan and telmisartan, have been identified as adiponectin inducers in essential hypertensive patients [10] and insulin-resistant animal models [39]. Clasen et al [39] recently demonstrated that ARB-induced adiponectin stimulation is likely to be mediated via the peroxisome proliferator–activated receptor γ (PPARγ) activation involving a posttranscriptional mechanism in 3T3-L1 adipocytes; the PPARγ antagonist markedly inhibited irbesartan-induced adiponectin expression. The olmesartan we used in this study is not a PPARγ-stimulated ARB; therefore, we think that the AT1R-induced mechanisms are more critical for ARB-induced adiponectin secretion.

Several lines of evidence support the theory that the simultaneous activation of AT2R counterbalances AT1R-mediated classic AII effectiveness. Because both AT1R and AT2R are present in adipocytes, we tried to determine whether blockade or stimulation of AT2R can change circulating adiponectin. Administrations of AT2R blocker PD123319 left the plasma adiponectin completely unchanged in the AII-infused rats. CGP42112A, an AT2R agonist, also failed to change the plasma adiponectin level. Collectively, we could not find any positive results implying that AT2R plays a role in regulating plasma adiponectin. Accordingly, adiponectin mRNA expression of adipose tissue was unchanged in the study of AT2R-deficeient mice by Yvan-Charvet et al [40].

In conclusion, long-term AII infusion decreased the circulating adiponectin concentration without affecting the gene expression in rats, and this may facilitate the development of insulin resistance. Angiotensin II type 1 receptor blocker ameliorated the AII-induced hypoadiponectinemia, whereas a modulation of AT2R exerted no effect on the plasma adiponectin, suggesting that the AII-induced hypoadiponectinemia took place via AT1R. Our results may provide a possible pathogenesis for insulin resistance associated with hypertension.

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