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Effect of Pioglitazone on Endothelial Dysfunction After Sirolimus-Eluting Stent Implantation

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Previous studies have demonstrated endothelial dysfunction after sirolimus-eluting stent (SES) implantation. The present study evaluated the effect of pioglitazone on endothelial dysfunction after SES implantation in nondiabetic patients. A total of 50 nondiabetic patients who had undergone SES implantation were randomly assigned to the pioglitazone group (n = 25) or the control group (n = 25). Endothelial function was estimated by measuring the coronary vasoreactivity in the reference segment within 15 mm proximal and distal to the SES in response to intracoronary acetylcholine infusion (10^{-8} and 10^{-7} mol/L) at 9 months of follow-up. Endothelium-independent vasomotion was assessed after an intracoronary bolus of nitroglycerin. Changes in the coronary diameter in response to 10^{-8} and 10^{-7} mol/L acetylcholine in the segment proximal to the SES were not significantly different between the pioglitazone and control groups. In contrast, in the segment distal to the SES, vasoconstrictions to 10^{-8} ($-3.0 \pm 2.8\%$ vs $-7.1 \pm 4.5\%$, $p < 0.01$) and 10^{-7} mol/L acetylcholine ($-6.2 \pm 8.0\%$ vs $-13.1 \pm 8.9\%$, $p < 0.01$) were attenuated in the pioglitazone group compared to the control group. Endothelium-independent vasodilation to nitrate did not differ between the 2 groups. Multivariate analysis showed that pioglitazone was an independent predictor improving endothelial dysfunction after SES implantation. In conclusion, pioglitazone might improve endothelial dysfunction after SES implantation in nondiabetic patients. © 2011 Elsevier Inc. All rights reserved. (Am J Cardiol 2011;108:214–219)

Previous studies have demonstrated abnormal endothelial function after sirolimus-eluting stent (SES) implantation.[1–5] Thiazolidinediones, peroxisome proliferator-activated receptor-γ agonists, are used to treat patients with type 2 diabetes mellitus as insulin-sensitizing agents.[6] It has been shown that thiazolidinediones improve endothelial dysfunction independently of glycemic control in animal models and human studies.[7–9] The present study evaluated the effect of pioglitazone on endothelial dysfunction after SES implantation in nondiabetic patients.

Methods

The institutional review boards at Chiba University Hospital approved the present open-label randomized study, and all patients provided written informed consent. The present study was registered at the University Hospital Medical Information Network Clinical Trials Registry in Japan, according to a statement from the International Committee of Medical Journal Editors in 2004, as registry identification University Hospital Medical Information Network Clinical Trials number 000000899.

A total of 50 nondiabetic patients who had been diagnosed with stable or unstable angina and treated with a SES for a de novo single lesion were enrolled. The nondiabetic state was determined by no history of diabetes mellitus and an assessment of the fasting plasma glucose with a level <126 mg/dl, and hemoglobin A1c level of <5.6%. The major criteria for exclusion were recent myocardial infarction (within the previous 48 hours), a history of coronary artery vasospasm, an ejection fraction of <40%, a target lesion in an unprotected left main coronary artery or a vessel with thrombus, severe calcified lesion, the presence of a >50% stenosis, except for a culprit lesion in the target vessel, a reference diameter of <2.5 or >4.0 mm, previous percutaneous coronary intervention in the target vessel, a contraindication to SES, and serious medical conditions. Intravascular ultrasound (IVUS)-guided stenting was performed in all patients. Stent implantation was performed according to current guidelines.[10] All stents were implanted to fully cover the lesion. Pre- and postdilation were performed, if necessary. Special care was taken to avoid geographic miss. In all patients, optimal stent expansion without residual stenosis and dissection of stent edge was confirmed by IVUS. After percutaneous coronary intervention, the patients continued a regimen of aspirin (100 mg/day) plus clopidogrel (75 mg/day) during 9 months of follow-up. The medications used before intervention were continued and were not changed during follow-up.

After the patients provided written informed consent, randomization was done with a sequentially numbered, opaque, sealed envelope in a 1:1 ratio. All patients were prospectively recruited from the Department of Cardiology at Chiba University Hospital. The patients were assigned to the pioglitazone group (n = 25) or the control group (n =

Department of Cardiovascular Science and Medicine, Chiba University Graduate School of Medicine, Chiba, Japan. Manuscript received January 23, 2011; manuscript received and accepted March 7, 2011.

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0002-9149/11/$ – see front matter
doi:10.1016/j.amjcard.2011.03.029

25). If allocated, we gave patients pioglitazone 15 mg/day for the initial 2 weeks and 30 mg/day thereafter. We saw patients monthly for the first 2 months and then every 2 months until follow-up angiography. The assessment of concentrations of fasting plasma glucose, hemoglobin A1c, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein (LDL) cholesterol, malondialdehyde LDL, high-sensitive C-reactive protein, high-molecular-weight adiponectin, and brain natriuretic peptide was performed at baseline and 9 months of follow-up.

Follow-up coronary angiography was performed 9 months after SES implantation. Endothelial function was estimated by measuring the coronary vasoreactivity in response to acetylcholine (Ach) infusion into the target coronary artery at 9 months of follow-up. All antianginal agents that influence vasomotor tone, including long-acting nitrates, calcium channel blockers, and β blockers, were withheld for $\geq$48 hours before follow-up angiography, except for sublingual nitroglycerin as needed. After baseline coronary angiography, serial Ach infusion (10^{-8} and 10^{-7} mol/L) into the target coronary artery through the 4Fr Judkins catheter for 2 minutes at each concentration was performed at an infusion rate of 2.0 ml/min.[2,3] Subsequently, endothelium-independent vasomotion was assessed after an intracoronary bolus of nitroglycerin (200 μg).

Using a computer-assisted, automated, edge-detection algorithm (CMS, MEDIS, Leiden, The Netherlands), off-line quantitative analysis of coronary angiography was performed by an experienced cardiologist (H.K.), who was unaware of the treatment group assignment.[11] Two orthogonal views with less foreshortening or without overlapping of side branches were selected. The minimum lumen diameter in the reference segments within 15 mm proximal and distal to the SES was measured. It was measured 3 times for each projection. The mean minimum lumen diameter was averaged for 2 projections. In addition, a reference angiographically normal segment in another vessel was measured, if the SES was implanted in the left coronary artery.[4] If the intervened vessel was the right coronary artery, an angiographically normal segment as far as possible from the stented vessel segment was measured as the reference. Changes in the coronary diameter in response to Ach and nitrate infusion are expressed as the percentage of changes versus the baseline angiographic findings. The primary end point was the change in the coronary diameter in response to 10^{-7} mol/L Ach in the segment distal to the SES. The secondary end points were the changes in coronary diameter in response to 10^{-8} mol/L Ach in a segment distal to the SES and in response to 10^{-8} and 10^{-7} mol/L Ach in the segment proximal to the SES.

IVUS was performed after administration of 200 μg of intracoronary nitroglycerin using the 40-MHz IVUS catheter (Boston Scientific, Natick, Massachusetts) with an automated pullback at 0.5 mm/s. According to the Clinical Expert Consensus Documents,[12] quantitative IVUS analysis was performed using commercially available planimetry software (echoPlaque, INDEC Systems, Mountain View, California).

In a previous study,[3] the change in the coronary diameter in response to 10^{-7} mol/L Ach in a segment distal to the SES was $-17.5 \pm 12.5\%$. We expected pioglitazone to achieve a 60% reduction in the change in coronary diameter in response to 10^{-7} mol/L Ach in a segment distal to the SES.[7] We estimated that 50 patients were required for a power of 80% and an α level of 0.05, assuming an angiographic follow-up rate of 92%.

Statistical analysis was performed with StatView, version 5.0, software (SAS Institute, Cary, North Carolina). Data are expressed as the mean $\pm$ SD or frequency (%). Comparisons within groups were performed by analysis of variance for repeated measurements. For intergroup comparisons, an unpaired Student's *t* test was applied. Categorical variables were analyzed using the chi-square test or Fish-

Table 1
Patient characteristics

Variable	Pioglitazone (n = 25)	Control (n = 25)
Age (years)	66.4 ± 7.9	66.8 ± 9.6
Men	22 (88%)	23 (92%)
Body mass index (kg/m^2)	23.0 ± 1.8	23.9 ± 3.9
Hypertension*	18 (72%)	20 (80%)
Hypercholesterolemia†	20 (80%)	22 (88%)
Current smoker	5 (20%)	3 (12%)
Previous myocardial infarction	4 (16%)	2 (8%)
Previous percutaneous coronary intervention	12 (48%)	11 (44%)
Clinical presentation at procedure		
Unstable angina pectoris	6 (24%)	5 (20%)
Stable angina pectoris	19 (76%)	20 (80%)
Left ventricular ejection fraction (%)	61.1 ± 6.5	59.7 ± 7.8
Medication		
Nitrate	10 (40%)	12 (48%)
Statin	21 (84%)	22 (88%)
Angiotensin-converting enzyme inhibitor or angiotensin receptor blocker	11 (44%)	7 (28%)
Calcium channel blocker	11 (44%)	13 (52%)
β Blocker	11 (44%)	9 (36%)
Eicosapentaenoic acid	0 (0%)	1 (4%)

* Defined as any patient receiving antihypertensive drugs or with systolic blood pressure $\geq$140 mm Hg and/or diastolic blood pressure $\geq$90 mm Hg.
† Defined as any patient receiving antihypercholesterelemic therapy or with untreated total cholesterol $\geq$220 mg/dl.

Table 2
Baseline angiographic and procedural characteristics

Variable	Pioglitazone (n = 25)	Control (n = 25)
Coronary artery		
Left anterior descending	14 (56%)	13 (52%)
Left circumflex	4 (16%)	7 (28%)
Right	7 (28%)	5 (20%)
Bifurcation	3 (12%)	7 (28%)
Moderate calcium	3 (12%)	4 (16%)
Chronic total occlusion	0 (0%)	2 (8%)
American Heart Association/American College of Cardiology type B2 or C	15 (60%)	18 (72%)
Stents/lesion	1.3 ± 0.5	1.4 ± 0.5
Stent diameter (mm)	3.0 ± 0.4	3.1 ± 0.4
Stent length (mm)	29.2 ± 11.9	33.4 ± 15.5
Maximum balloon diameter (mm)	3.1 ± 0.5	3.2 ± 0.4
Maximum balloon inflation (atm)	17.7 ± 3.1	17.5 ± 3.6

Table 3
Baseline and follow-up laboratory data and blood pressure

Variable	Pioglitazone (n = 25)	Control (n = 25)	p Value
Fasting plasma glucose (mg/dl)			
Baseline	97 ± 6	97 ± 7	0.80
Follow-up	100 ± 15	98 ± 12	0.62
Δ (follow-up – baseline)	3.3 ± 16	0.9 ± 9.5	0.54
Hemoglobin A1c (%)			
Baseline	5.2 ± 0.3	5.2 ± 0.3	0.89
Follow-up	5.3 ± 0.3	5.3 ± 0.3	0.57
Δ (follow-up – baseline)	0.1 ± 0.2	0.1 ± 0.2	0.19
Triglycerides (mg/dl)			
Baseline	146 ± 61	194 ± 135	0.12
Follow-up	146 ± 187	159 ± 81	0.75
Δ (follow-up – baseline)	−0.4 ± 182	−35 ± 94	0.41
High-density lipoprotein cholesterol (mg/dl)			
Baseline	53 ± 11	50 ± 15	0.33
Follow-up	58 ± 13	56 ± 10	0.48
Δ (follow-up – baseline)	4.9 ± 9.5	6.3 ± 12	0.66
Low-density lipoprotein cholesterol (mg/dl)			
Baseline	114 ± 32	107 ± 28	0.17
Follow-up	93 ± 18	93 ± 24	0.95
Δ (follow-up – baseline)	−22 ± 33	−9.3 ± 31	0.19
Malondialdehyde low-density lipoprotein (U/L)			
Baseline	113 ± 43	99 ± 35	0.21
Follow-up	88 ± 37	96 ± 34	0.42
Δ (follow-up – baseline)	−26 ± 39	−3.1 ± 25	0.02
High-sensitivity C-reactive protein (mg/L)			
Baseline	3.4 ± 7.6	2.1 ± 2.7	0.45
Follow-up	0.4 ± 0.4	0.7 ± 0.7	0.07
Δ (follow-up – baseline)	−3.0 ± 7.7	−1.5 ± 2.8	0.35
High-molecular-weight adiponectin (μg/ml)			
Baseline	3.2 ± 1.7	4.6 ± 3.5	0.08
Follow-up	14 ± 10	4.8 ± 3.9	<0.001
Δ (follow-up – baseline)	10 ± 9	0.2 ± 2.1	<0.001
Brain natriuretic peptide (pg/ml)			
Baseline	32 ± 27	54 ± 62	0.13
Follow-up	41 ± 31	43 ± 49	0.89
Δ (follow-up – baseline)	7.8 ± 33	−11 ± 53	0.16
Systolic blood pressure (mm Hg)			
Baseline	122 ± 8	122 ± 8	0.86
Follow-up	126 ± 11	126 ± 11	0.98
Δ (follow-up – baseline)	4.1 ± 11	3.8 ± 10	0.92
Diastolic blood pressure (mm Hg)			
Baseline	72 ± 7	70 ± 7	0.26
Follow-up	71 ± 10	72 ± 8	0.61
Δ (follow-up – baseline)	−1.6 ± 11	2.1 ± 9.1	0.20

er's exact test. A value of $p < 0.05$ was considered significant. Univariate analysis was performed using linear regression analysis of rank-transformed outcomes. Including only variables with a p value of <0.1 on univariate analysis, multivariate analysis was performed using multiple linear regression analysis of rank-transformed outcomes.

Results

The baseline patient, angiographic, and procedural characteristics were similar between the 2 groups (Tables 1 and 2). The baseline and follow-up laboratory data and blood pressure are listed in Table 3. No significant difference was found in the IVUS measurements between the 2 groups (Table 4).

Table 4
Intravascular ultrasound analysis

	Pioglitazone (n = 25)	Control (n = 25)	p Value
Proximal to sirolimus-eluting stent			
External elastic membrane area (mm^2)	16.0 ± 5.2	17.3 ± 4.5	0.38
Lumen area (mm^2)	9.9 ± 4.0	10.4 ± 2.6	0.65
Plaque area (mm^2)	6.1 ± 2.7	6.9 ± 2.6	0.31
Plaque burden (%)	37.9 ± 13.0	38.8 ± 9.2	0.79
Distal to sirolimus-eluting stent			
External elastic membrane area (mm^2)	10.4 ± 5.8	10.6 ± 4.2	0.90
Lumen area (mm^2)	7.4 ± 3.8	7.4 ± 2.6	0.96
Plaque area (mm^2)	3.0 ± 2.1	3.2 ± 1.9	0.68
Plaque burden (%)	27.2 ± 6.3	28.7 ± 8.0	0.48

No adverse cardiac events occurred during the initial hospitalization and follow-up. Side effects of pioglitazone were observed in 2 patients (8.0%; moderate pretibial pitting edema and gastrointestinal disorder). In both, the dose of pioglitazone was decreased from 30 to 15 mg/day and the side effects disappeared. All patients underwent 9-month follow-up angiography at 315 ± 33 days for the pioglitazone group and 317 ± 54 days for the control group ($p = 0.84$). No stent edge restenosis occurred in either group. One patient in the control group developed focal restenosis of 52% severity at the body of the stent. No patient had target lesion revascularization.

No significant difference was found in vasomotor reactivity in the reference angiographically normal segment between the 2 groups (Figure 1). No significant difference was found between the pioglitazone and control groups in terms of the baseline reference segment diameter proximal (2.22 ± 0.45 vs 2.49 ± 0.54 mm, $p = 0.07$) and distal (1.67 ± 0.44 vs 1.77 ± 0.42 mm, $p = 0.42$) to the SES. They were smaller than the SES diameter, because all vasodilators were withheld before the procedure. In both groups, Ach infusion induced significant vasoconstriction in the segment either proximal ($p < 0.01$) or distal ($p < 0.01$) to the SES. The changes in the coronary diameter in response to 10^{-8} (−2.3 ± 3.5% vs −3.7 ± 3.7%, $p = 0.19$) and 10^{-7} mol/L Ach (−5.7 ± 8.4% vs −8.5 ± 8.9%, $p = 0.29$) in the segment proximal to the SES were not significantly different between the pioglitazone and control groups (Figure 1). In contrast, in the segment distal to the SES, vasoconstriction to 10^{-8} (−3.0 ± 2.8% vs −7.1 ± 4.5%, $p < 0.01$) and 10^{-7} mol/L Ach (−6.2 ± 8.0% vs −13.1 ± 8.9%, $p < 0.01$) was attenuated in the pioglitazone group compared to the control group (Figure 1). It was observed even after excluding the patient who had in-stent restenosis (data not shown). Endothelium-independent vasodilation to nitrate did not differ between the 2 groups. The univariate and multivariate predictors of improving endothelial dysfunction in response to Ach (10^{-7} mol/L) in the segment distal to the SES are demonstrated in Table 5. Multivariate analysis showed that pioglitazone was an independent predictor of improving endothelial dysfunction after SES implantation.

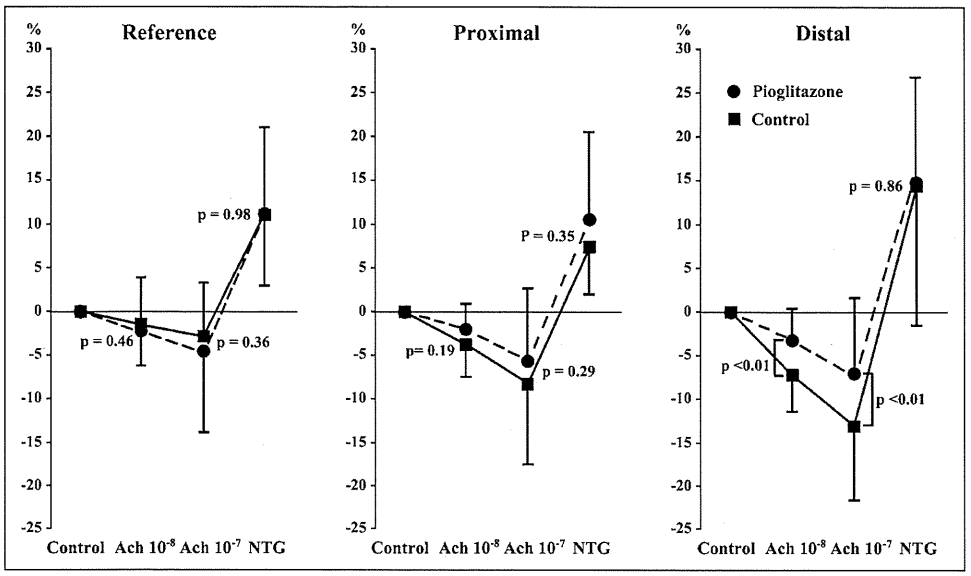

Figure 1. Changes in coronary diameter in response to Ach and nitrate infusion expressed as percentage of changes versus baseline angiograms. p Values indicate difference between pioglitazone and control groups.

Table 5
Predictors of Improving Endothelial Dysfunction* After SES Implantation

	Univariate		Multivariate	
	β Coeff. (95% CI)	p Value	β Coeff. (95% CI)	p Value
Pioglitazone	6.9 (2.1 to 11.6)	0.005	5.4 (0.4 to 10.4)	0.03
Stent length	−0.2 (−0.4 to −0.05)	0.01	−0.2 (−0.5 to 0.08)	0.14
Baseline LDL cholesterol	0.07 (−0.01 to 0.1)	0.08	0.06 (−0.02 to 0.1)	0.15
ACEI or ARB	−4.6 (−9.8 to 0.5)	0.08	−3.7 (−9.0 to 1.5)	0.16
Age	−0.3 (−0.5 to 0.02)	0.07	−0.2 (−0.5 to 0.1)	0.23
Moderate or severe calcification	−7.2 (−14.0 to −0.4)	0.04	−3.9 (−10.5 to 2.7)	0.24
Baseline HMW adiponectin	−0.3 (−0.5 to 0.02)	0.02	−0.3 (−1.2 to 0.6)	0.48
Baseline diastolic blood pressure	0.3 (−0.05 to 0.7)	0.09	−0.1 (−0.5 to 0.2)	0.49
Stents/lesion	−4.8 (−10.0 to 0.4)	0.07	1.4 (−7.8 to 10.6)	0.76

* Endothelial dysfunction in response to acetylcholine (10^{-7}mol/L) in segment distal to SES.

ACEI = angiotensin-converting enzyme inhibitor; ARB = angiotensin receptor blocker; HMW = high molecular weight; LDL = low-density lipoprotein; SES = sirolimus-eluting stent.

Discussion

The present study has shown that pioglitazone improved endothelial dysfunction after SES implantation. Several recent studies have demonstrated abnormal endothelial function after SES implantation[1–5] that might be associated with adverse cardiac events after SES implantation.[13] Togni et al[1] performed quantitative coronary angiography at rest and during supine bicycle exercise testing 6 months after stenting. Exercise-induced paradoxical coronary vasoconstriction was observed in the vessel segment adjacent to the SES but not with bare metal stents.[1] Hofma et al[2] demonstrated significant vasoconstriction in segments distal to the SES but not with bare metal stents after Ach infusion at 6 months of follow-up. Endothelial cells produce many vasoactive substances that maintain vascular homeostasis and normal vasomotor tone.[5] Nitric oxide is a key factor generated by endothelial cells. It is the main determinant of basal vascular smooth muscle tone and opposes the actions of potent endothelium-derived contracting factors such as angiotensin II and endothelin 1.[14,15] Furthermore, it inhibits platelet activation. Impairment of the bioactivity or bioavailability of nitric oxide and/or an imbalance between endothelium-derived relaxing and contracting factors is crucial in the mechanisms of endothelial dysfunction.[16]

Thiazolidinediones, peroxisome proliferator-activated receptor-γ agonists, are used to treat patients with type 2 diabetes as insulin-sensitizing agents.[6] The PROspective pioglitAzone Clinical Trial In macroVascular Events (PROactive) study is a prospective, randomized controlled trial of diabetic patients with macrovascular disease that evaluated the effects of pioglitazone on cardiovascular events.[17] The composite secondary end point of all-cause mortality, nonfatal myocardial infarction, and stroke was lower in the pioglitazone group than in the placebo group.[17] The Pioglitazone Effect on Regression of Intravascular Sonographic Coronary Obstruction Prospective Evaluation (PERISCOPE) trial demonstrated that treatment with pioglitazone resulted in a significantly lower rate of progression of cor-

onary atherosclerosis in diabetic patients compared to glimepiride.[18]

Previous studies have shown that thiazolidinediones have pleiotropic effects, including anti-inflammatory and antiatherogenic vascular effects.[19,20] Furthermore, it has been reported that thiazolidinediones improve endothelial dysfunction in animal models.[7] Clinical studies have shown a beneficial effect of thiazolidinediones on peripheral vascular endothelial function in diabetic patients.[8,9] Recently, Wöhrle et al[9] have demonstrated a beneficial effect of pioglitazone on coronary endothelial function in nondiabetic patients. Quantitative coronary angiography after intracoronary infusion of Ach showed a decrease ($-7.6 \pm 2.4\%$) in the lumen area between the baseline and 6-month follow-up examinations in the control group. In contrast, pioglitazone treatment increased in lumen area ($1.8 \pm 2.0\%$; $p < 0.008$ vs the control group). In the present study, the beneficial effect of pioglitazone was observed in the segment distal to the SES but not the segment proximal to the SES. Sirolimus from the SES moves with blood flow. Thus, endothelial dysfunction might be more pronounced in segments distal to the SES than proximal to the SES. The beneficial effect of pioglitazone might be observed in more impaired segments.

In the present study, no significant difference was found in the fasting plasma glucose and hemoglobin A1c levels between the 2 groups. This finding suggests that the beneficial effect of pioglitazone on endothelial function is independent of its glycemic effect. Pioglitazone treatment decreased malondialdehyde LDL and increased high-molecular-weight adiponectin. Previous studies have shown that oxidized LDL cholesterol induced endothelial dysfunction.[21] In contrast, high-molecular-weight adiponectin has been shown to be an independent factor improving endothelial function.[22] Previous studies[18] have shown that pioglitazone decreases high-sensitivity C-reactive protein. Pioglitazone stimulates the release of nitric oxide from endothelial cells.[23] It inhibits the expression of vascular cell adhesion molecule and intercellular adhesion molecule and reduces plasma tumor necrosis factor-α, monocyte chemoattractant protein-1, plasminogen activator inhibitor type 1, and C-reactive protein.[24,25] These might have a beneficial effect on endothelial function. Endothelial progenitor cells play an important role in endothelial repair. Sirolimus inhibits proliferation, migration, and differentiation of endothelial progenitor cells.[26] In contrast, the beneficial effect of thiazolidinediones on the circulating levels and function of endothelial progenitor cells have been demonstrated.[27] It could be another possible mechanism for improving endothelial function.

Serious concerns exists about rosiglitazone.[28] However, a meta-analysis of randomized trials showed that, compared to control therapy, pioglitazone was associated with a significantly lower risk of death, myocardial infarction, or stroke among a diverse population of patients with diabetes.[29] Various peroxisome proliferators activated receptor-γ agonists can yield markedly different patterns of gene modulation, resulting in complex and largely unknown differences in effects on the metabolic pathways.[29] Compared to rosiglitazone, pioglitazone produces greater reductions in serum triglycerides and increases in high-density lipoprotein cholesterol levels.[29] It could be that pioglitazone has a different safety and efficacy profile than rosiglitazone.

The present study had some limitations. First, the present study was a single-center study, and the number of enrolled patients was relatively small. However, no patients stopped taking pioglitazone, and all patients underwent follow-up angiography and evaluation of endothelial function. Second, we did not evaluate the baseline endothelial function. Thus, it is unclear whether patients with abnormal endothelial function before SES implantation were included. However, the percentage of changes in the coronary diameter from baseline to nitrate infusion were not different between the pioglitazone and control groups. No significant difference was found in vasomotor reactivity in the reference angiographically normal segment between the 2 groups. Furthermore, the doses of intracoronary Ach infusion were much lower compared to those for provocation of vasospasm. Third, we enrolled patients undergoing SES implantation. Thus, it is unknown whether pioglitazone improves endothelial function after implantation of different types of drug-eluting stents.

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THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 286, NO. 24, pp. 21458–21465, June 17, 2011

Angiotensin II Type 1 Receptor Signaling Regulates Feeding Behavior through Anorexigenic Corticotropin-releasing Hormone in Hypothalamus*[S]

Received for publication, October 7, 2010, and in revised form, March 3, 2011 Published, JBC Papers in Press, April 27, 2011, DOI 10.1074/jbc.M110.192260

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The activation of renin-angiotensin system contributes to the development of metabolic syndrome and diabetes as well as hypertension. However, it remains undetermined how renin-angiotensin system is implicated in feeding behavior. Here, we show that angiotensin II type 1 (AT_1) receptor signaling regulates the hypothalamic neurocircuit that is involved in the control of food intake. Compared with wild-type *Agtr1a*$^{+/+}$ mice, AT_1 receptor knock-out (*Agtr1a*$^{-/-}$) mice were hyperphagic and obese with increased adiposity on an *ad libitum* diet, whereas *Agtr1a*$^{-/-}$ mice were lean with decreased adiposity on a pair-fed diet. In the hypothalamus, mRNA levels of anorexigenic neuropeptide corticotropin-releasing hormone (*Crh*) were lower in *Agtr1a*$^{-/-}$ mice than in *Agtr1a*$^{+/+}$ mice both on an *ad libitum* and pair-fed diet. Furthermore, intracerebroventricular administration of CRH suppressed food intake both in *Agtr1a*$^{+/+}$ and *Agtr1a*$^{-/-}$ mice. In addition, the *Crh* gene promoter was significantly transactivated via the cAMP-responsive element by angiotensin II stimulation. These results thus demonstrate that central AT_1 receptor signaling plays a homeostatic role in the regulation of food intake by maintaining gene expression of *Crh* in hypothalamus and suggest a therapeutic potential of central AT_1 receptor blockade in feeding disorders.

Maintaining energy homeostasis through regulated food intake and body weight is fundamental for survival. However, >1 billion people are now classified as obese or overweight, and the prevalence of these conditions is increasing rapidly (1). As a major risk factor for cardiovascular diseases and diabetes, obesity has become a leading public health threat and economic burden worldwide. On the other hand, weight loss is a serious and occasionally life-threatening problem in patients with anorexia nervosa and in cachectic patients suffering from chronic obstructive pulmonary disease and cancer. Although environmental and lifestyle factors contribute to body weight gain or loss, impaired regulation of food intake also underlies the pathogenesis of these eating disorders (2). Recent studies have demonstrated that feeding behavior is under control of the central neural circuit involving the hypothalamus. Hypothalamus contains several nuclei that exert homeostatic control of food intake through orexigenic and anorexigenic signals (2).

The renin-angiotensin system (RAS)[3] plays a crucial role in the physiological control of blood pressure and fluid balance and also participates in the pathological processes in the development of cardiovascular and metabolic diseases. Although the activation of RAS in peripheral organs contributes to the development of obesity and the metabolic syndrome (3–5), it remains undetermined how RAS is implicated in feeding behavior. The effects of angiotensin II (Ang II), a pivotal bioactive molecule of RAS, are mainly mediated through the type 1 (AT_1) receptor (6). In rodents, AT_1 receptor consists of two subtypes (AT_{1a} and AT_{1b}), but AT_{1a} receptor is predominantly expressed and functionally important in most organs including the heart, blood vessels, kidney, adrenal glands, brain, and adipose tissues (7, 8). Feeding behavior is regulated by multiple orexigenic peptides such as neuropeptide Y (NPY), agouti-related protein (AgRP), orexin, and melanin-concentrating hormone (MCH), and anorexigenic peptides such as corticotropin-releasing hormone (CRH), pro-opiomelanocortin (POMC), and cocaine- and amphetamine-regulated transcript (CART) in hypothalamus (2). The AT_{1a} receptor is expressed in hypothalamus, including paraventricular nucleus (PVN), lateral hypothalamic area, perifornical nucleus, and retrochiasmatic area

* This work was supported in part by grants from Japan Society for the Promotion of Science (KAKENHI 20390218 and 21229010); Health and Labor Sciences Research Grants (to I. K. and H. A.) and grants from Takeda Science Foundation, Astellas Foundation for Research on Metabolic Disorders, The Uehara Memorial Foundation, The Ichiro Kanehara Foundation, Mochida Memorial Foundation for Medical and Pharmaceutical Research, and the Suzuken Memorial Foundation (to H. A.); and a Research Fellowship from the Japan Association for the Advancement of Medical Equipment (to R. Y.).

[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental "Experimental Procedures" and Figs. S1–S5.

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[3] The abbreviations used are: RAS, renin-angiotensin system; AgRP, agouti-related protein; AngII, angiotensin II; AT_1 receptor, AngII type 1 receptor; CART, cocaine- and amphetamine-regulated transcript; CRE, cAMP-responsive element; CREB, CRE-binding protein; CRH, corticotropin-releasing hormone; Luc, luciferase; DN, dominant-negative; i.c.v., intracerebroventricular; MCH, melanin-concentrating hormone; NPY, neuropeptide Y; POMC, pro-opiomelanocortin; PVN, paraventricular nucleus.

(9), but the regulatory role of AT_1 receptor signaling in the hypothalamic neuronal circuit remains precisely unknown.

In the present study, we demonstrate that AT_{1a} receptor knock-out (*Agtr1a*$^{-/-}$) mice were hyperphagic and obese on an *ad libitum* diet, but not on a pair-fed diet, compared with wild-type *Agtr1a*$^{+/+}$ mice and that central AT_1 receptor signaling controls food intake by regulating anorexigenic *Crh* gene expression in hypothalamus.

EXPERIMENTAL PROCEDURES

Animals—Generation of *Agtr1a*$^{-/-}$ mice on the C57BL/6J Jcl background has been described previously (10). Male mice were used in this study. We maintained mice in individual cages under a temperature- and humidity-controlled condition with a 12 h light-dark cycle. Mice were allowed free access to water and standard chow (343.1 kcal/100 g; crude protein 25.1%; crude fat 4.8%; crude fiber 4.2%; crude ash 6.7%; nitrogen-free extract 50.0%; Clea Japan, Tokyo, Japan) on an *ad libitum*-feeding. For pair-feeding experiments, *Agtr1a*$^{-/-}$ mice were restricted to the amount of food consumed by *ad libitum*-fed *Agtr1a*$^{+/+}$ mice, as described previously (11). Pair feeding was started on mice at 5 weeks of age, and continued for a period of 11 weeks. All of the experimental protocols were approved by the Institutional Animal Care and Use Committee of Chiba University.

Cold Exposure Test—Mice were kept at 4 °C for 60 min and then kept at room temperature for 30 min. Rectal body temperatures were measured with a thermometer (BWT-100; Bio Research Center, Nagoya, Japan) at 0, 60, 75, and 90 min during the experiment.

Blood and Urine Analysis—Blood glucose levels were determined by using ACCU-CHEK Blood Glucose Meter (Roche Diagnostics, Basel, Switzerland). For a glucose tolerance test, a glucose load was injected i.p. (1 g/kg body weight) after a 16 h fast. Blood glucose concentrations were measured at 0 (before), 30, 60, 90, and 120 min after the injection. For insulin tolerance test, insulin (Humulin R; Eli Lilly, Indianapolis, IN) was injected i.p. (1 unit/kg body weight) after a 1-h fast. Blood glucose concentrations were measured at 0 (before), 15, 30, 45, and 60 min after the injection. Serum leptin concentrations were assayed by using mouse leptin quantikine ELISA kit (R&D Systems) according to the manufacturer's protocol. Urine concentrations of catecholamines were measured by HPLC in the laboratory of SRL, Inc. (Tokyo, Japan).

Histological Analysis—The liver and epididymal fat were excised, immediately fixed in 10% neutralized formalin, and embedded in paraffin. Sections at 5 μm were stained with hematoxylin and eosin.

In Situ Hybridization—The mice were decapitated, and the brains were rapidly removed and frozen. Frozen sections at 12 μm were used for *in situ* hybridization, as described previously (12). Antisense probes were 3′-end labeled with ^{35}S by using oligonucleotides complementary to mRNAs of *Npy*, *Agrp*, *Hcrt*, *Pmch*, *Crh*, *Pomc*, and *Cartpt*. A semi-quantitative image analysis was performed with the MCID Image Analysis System (Imaging Research, St. Catharines, Ontario, Canada).

Intracerebroventricular Administration of CRH—The mice were anesthetized with inhalation of diethyl ether. Vehicle or 0.2 μg of CRH (Sigma-Aldrich, St. Louis, MO) in a total volume of 1 μl was administered intracerebroventricularly (i.c.v.) to the anesthetized mice by using a Hamilton syringe with a catheter PH03 (Hamilton Company, Reno, NV) into the right lateral ventricle using the coordinates: 0.5 mm caudal to bregma, 1 mm lateral to sagital suture, and 2 mm in depth.

Luciferase Assays—HEK293 cells expressing the AT_1 receptor were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and were starved under a serum-free condition for 48 h before stimulation with 10^{-7} M of Ang II (Sigma-Aldrich) (13). The *Crh* luciferase reporter plasmids, with or without the expression vector for dominant-negative form of cAMP-responsive element (CRE)-binding protein (DN-CREB), was transfected by the FuGENE 6 Transfection Regent (Roche Diagnostics), according to the manufacturer's instructions. pRL-SV40 (Promega, Madison, WI) was co-transfected as an internal control. Luciferase activities were measured 24 h after Ang II stimulation by using the Dual-Luciferase reporter assay system (Promega). Experiments were repeated at least three times in triplicate, and representative data are shown. The *Crh* luciferase reporter plasmids and the expression vector for dominant-negative form of CRE-binding protein (DN-CREB) (14) were a generous gift from Dr. Y. Iwasaki (Kochi University).

Statistics—The results are expressed as the mean ± S.E. Differences in measured values were evaluated with an analysis of variance using Fisher's *t* test and an unpaired Student's *t* test. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Agtr1a$^{-/-}$ *Mice Are Obese and Hyperphagic under ad Libitum Feeding*—We first compared the body weight between male *Agtr1a*$^{+/+}$ and *Agtr1a*$^{-/-}$ mice on the C57BL/6 background. When maintained *ad libitum* on standard laboratory chow, *Agtr1a*$^{-/-}$ mice were significantly heavier than *Agtr1a*$^{+/+}$ mice after 3 weeks of age and weighed 11.5 ± 2.9% more than *Agtr1a*$^{+/+}$ mice at 24 weeks of age (Fig. 1, *A–C*). Although the tibial length was indistinguishable, liver and epididymal fat were significantly heavier in *Agtr1a*$^{-/-}$ mice (Fig. 1*C*). Because an excess of body weight results from an imbalance between food intake and energy expenditure, we compared the food intake between *Agtr1a*$^{+/+}$ and *Agtr1a*$^{-/-}$ mice. The daily food intake of *Agtr1a*$^{-/-}$ mice was significantly more throughout the course of observation, than that of *Agtr1a*$^{+/+}$ mice (Fig. 1*D*).

On the other hand, i.p. glucose tolerance test and insulin tolerance test revealed that the glucose disposal and the hypoglycemic effect of insulin were pronounced in *Agtr1a*$^{-/-}$ mice compared with *Agtr1a*$^{+/+}$ mice (Fig. 2). These results suggest that *Agtr1a*$^{-/-}$ mice have better glucose tolerance and insulin sensitivity despite increased food intake, body weight, and adiposity. Although the core body temperatures were comparable under basal conditions (*Agtr1a*$^{+/+}$, 38.5 ± 0.1 °C; *Agtr1a*$^{-/-}$, 38.4 ± 0.1 °C; $n = 8$ per group; $p = 0.234$), *Agtr1a*$^{-/-}$ mice showed enhanced thermogenic response after cold exposure, compared with *Agtr1a*$^{+/+}$ mice (Fig. 3), suggesting that energy expenditure was increased in *Agtr1a*$^{-/-}$ mice, as reported previously (15). In addition, clinical and experimental studies have

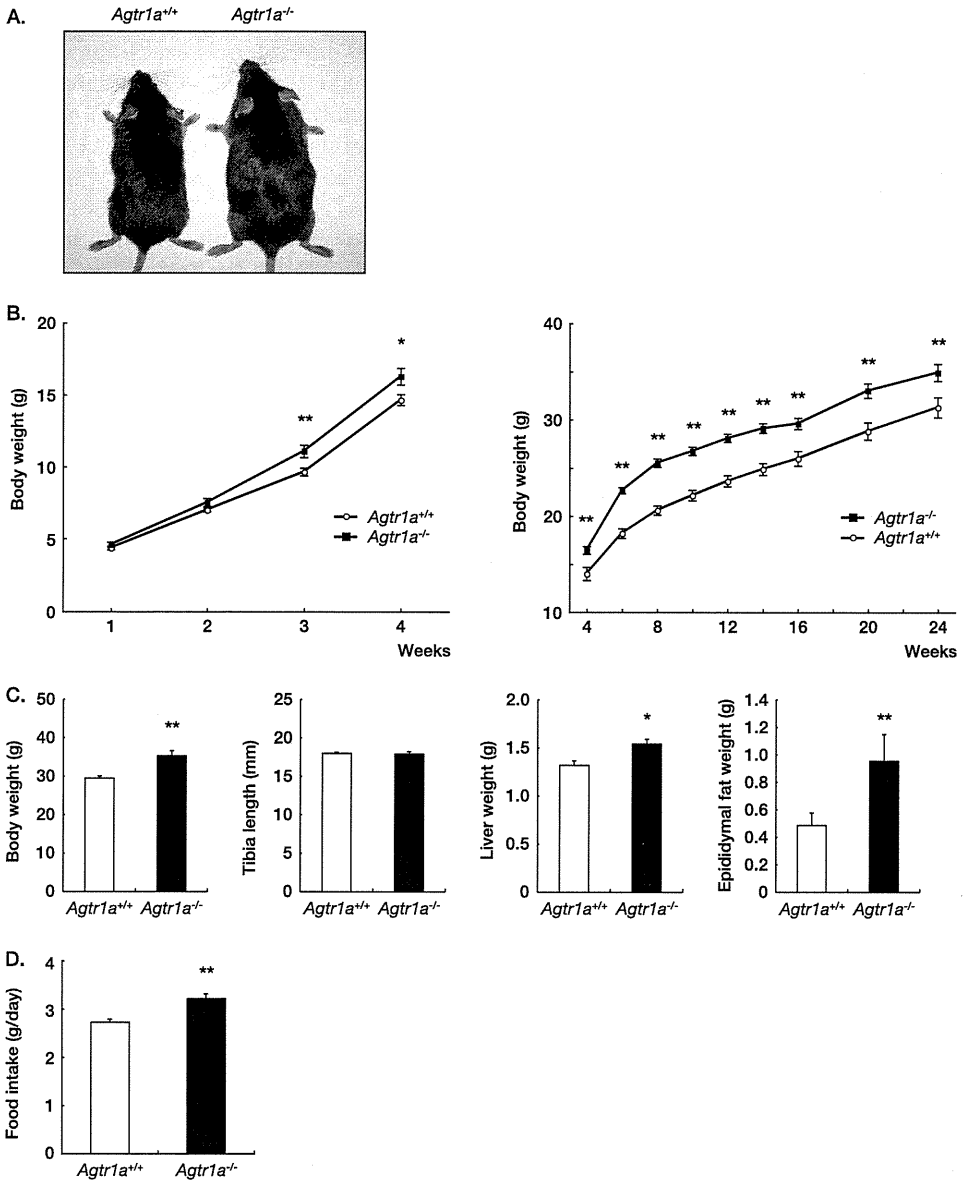

FIGURE 1. **Agtr1a$^{-/-}$ mice are obese and hyperphagic on a standard diet.** *A*, gross appearance of male *Agtr1a*$^{+/+}$ and *Agtr1a*$^{-/-}$ mice at 16 weeks of age. *B*, growth curves of male *Agtr1a*$^{+/+}$ ($n = 20$) and *Agtr1a*$^{-/-}$ ($n = 25$) mice. **, $p < 0.01$. *C*, body and organ weight, and tibia length of male *Agtr1a*$^{+/+}$ ($n = 9$) and *Agtr1a*$^{-/-}$ ($n = 9$) mice at 24 weeks of age. *, $p < 0.05$; **, $p < 0.01$. *D*, daily food intake of *ad libitum*-fed male *Agtr1a*$^{+/+}$ ($n = 20$) and *Agtr1a*$^{-/-}$ ($n = 25$) mice at 16 weeks of age. **, $p < 0.01$.

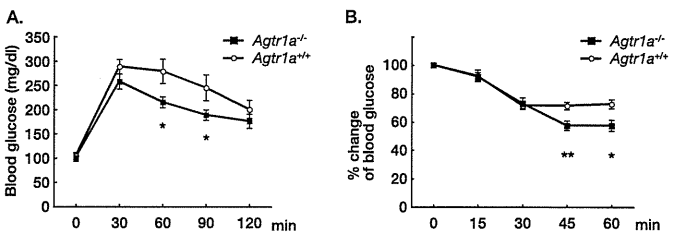

FIGURE 2. **Agtr1a$^{-/-}$ mice show lower glucose concentrations than *Agtr1a*$^{+/+}$ mice after i.p. injection of glucose or insulin.** *A*, glucose tolerance test (*Agtr1a*$^{+/+}$, $n = 8$; *Agtr1a*$^{-/-}$, $n = 11$) at 24 weeks of age. *, $p < 0.05$. *B*, insulin tolerance test (*Agtr1a*$^{+/+}$, $n = 8$; *Agtr1a*$^{-/-}$, $n = 9$) at 24 weeks of age. *, $p < 0.05$; **, $p < 0.01$.

demonstrated that pharmacological inhibition of RAS improves insulin sensitivity in part through increased glucose uptake in skeletal muscle (3). We suppose that the beneficial effect of *Agtr1a* deficiency on glucose metabolism overcomes the detrimental effect of increased body weight and adiposity in *Agtr1a*$^{-/-}$ mice.

Agtr1a$^{-/-}$ *Mice Weigh Less Than Agtr1a*$^{+/+}$ *Mice under Pair feeding*—Next, we examined the relevance of hyperphagia to increased body weight gain and adiposity in *Agtr1a*$^{-/-}$ mice. For this purpose, we restricted 5-week-old *Agtr1a*$^{-/-}$ mice to the amount of food consumed by *ad libitum*-fed *Agtr1a*$^{+/+}$ littermate mice and continued pair-feeding for 11 weeks. On a pair-fed diet, the body weight gain of *Agtr1a*$^{-/-}$ mice was less than that of *Agtr1a*$^{+/+}$ mice, and *Agtr1a*$^{-/-}$ mice weighed 11.9 ± 2.3% less than *Agtr1a*$^{+/+}$ mice at 16 weeks of age (Fig. 4, *A* and *B*). In parallel with body weight, the weights of liver and epididymal fat in pair-fed *Agtr1a*$^{-/-}$ mice were comparable with or less than those of *Agtr1a*$^{+/+}$ mice (liver: $p = 0.806$; epididymal fat; $p < 0.01$, Fig. 4*B*). Histological analysis revealed that hepatic steatosis and adipocyte hypertrophy were promi-

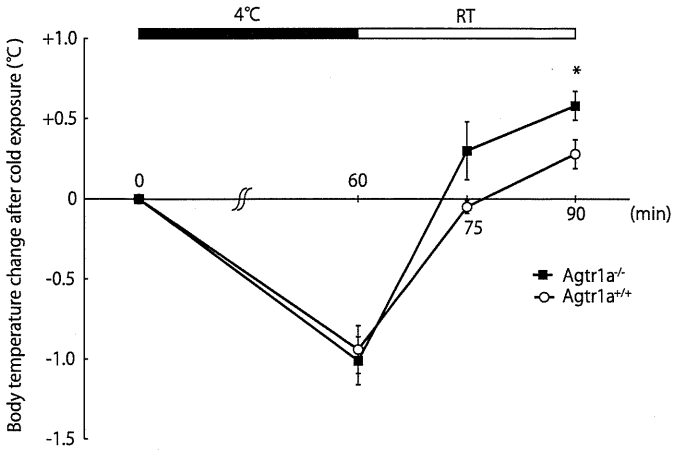

FIGURE 3. **$Agtr1a^{-/-}$ mice show enhanced thermogenic response after cold exposure.** Changes in body temperature after cold exposure in $Agtr1a^{+/+}$ ($n = 8$) and $Agtr1a^{-/-}$ ($n = 8$) mice on an *ad libitum* diet at 16 weeks of age. *, $p < 0.05$. *RT*, room temperature.

nent in *ad libitum*-fed $Agtr1a^{-/-}$ mice, but not in pair-fed $Agtr1a^{-/-}$ mice (Fig. 4*C*). In general, weight gain and fat content are associated with increased levels of leptin (16). The serum leptin concentrations of $Agtr1a^{-/-}$ mice were significantly higher on an *ad libitum*-fed diet, but lower on a pair-fed diet, than those of $Agtr1a^{+/+}$ mice (Fig. 4*D*). In pair-fed $Agtr1a^{-/-}$ mice, sympathetic activation may induce higher levels of energy expenditure, resulting in less body weight. Supporting this hypothesis, pair-fed $Agtr1a^{-/-}$ mice exhibited higher levels of daily urinary catecholamine than $Agtr1a^{+/+}$ mice (Table 1). Therefore, we suppose that an excess of food intake is causative to increased body weight and adiposity in *ad libitum*-fed $Agtr1a^{-/-}$ mice.

Hypothalamic Crh Expression Is Decreased in ad Libitum- and Pair-fed $Agtr1a^{-/-}$ Mice—Feeding behavior is regulated by multiple orexigenic and anorexigenic peptides in hypothalamus (2). To elucidate the mechanism underlying hyperphagia in $Agtr1a^{-/-}$ mice, we performed semi-quantitative *in situ* hybridization analysis of appetite-related peptide genes in the hypothalamus (2). Despite hyperphagia, the expression levels of orexigenic *Npy* and *Agrp* in arcuate nucleus and *Hcrt* in lateral hypotharamic area were lower in *ad libitum*-fed $Agtr1a^{-/-}$ mice than in $Agtr1a^{+/+}$ mice (Fig. 5*A*). In contrast, the expression levels of *Npy* and *Agrp* were higher in pair-fed $Agtr1a^{-/-}$ mice (Fig. 5*B*). It has been reported that *Npy* and *Agrp* mRNA expression are suppressed in the state of high leptin levels and enhanced in the state of low leptin levels (17–19). In $Agtr1a^{-/-}$ mice, the *Npy* and *Agrp* expressions were in inverse proportion to the levels of leptin concentration and adiposity. The expression levels of anorexigenic *Pomc* and *Cartpt* were decreased in $Agtr1a^{-/-}$ mice, which were statistically indistinguishable from those in $Agtr1a^{+/+}$ mice. Notably, the expression levels of anorexigenic *Crh* in PVN were significantly decreased in both *ad libitum*- or pair-fed $Agtr1a^{-/-}$ mice, compared with $Agtr1a^{+/+}$ mice (Fig. 5, *A* and *B*). It has been reported that the *Crh* expression is stimulated by leptin and that a CRH antagonist attenuates the leptin-induced reduction in food intake and body weight (20, 21). Therefore, these results raised the possibility that a down-regulation of anorexigenic *Crh*, occurring irrespective of the leptin levels, was the cause of hyperphagia in $Agtr1a^{-/-}$ mice.

i.c.v. Injection of CRH Reduces Food Intake in $Agtr1a^{-/-}$ Mice—CRH is a major regulator of the hypothalamo-pituitary-adrenocortical axis and also functions as an endogenous inhibitor of food intake (2). Indeed, i.c.v. injection of CRH reduced spontaneous or deprivation-induced feeding (22), but $Crh^{-/-}$ mice did not exhibit an increase in food intake under physiological conditions (23). In general, appetite in animals and humans is closely related to the glucocorticoid levels. Although $Crh^{-/-}$ mice showed a markedly low corticosterone levels (23), the serum corticosterone concentrations were not significantly different between $Agtr1a^{+/+}$ and $Agtr1a^{-/-}$ mice on an *ad libitum* diet (supplemental Fig. S1). Interestingly, it has been reported that the food intake of $Crh^{-/-}$ mice was significantly higher than that of $Crh^{+/+}$ mice, when the corticosterone levels were adjusted to the equal levels through adrenalectomy and corticosterone replacement (24). We speculate that down-regulation of *Crh* in $Agtr1a^{-/-}$ mice activates downstream satiety and reward circuits to increase food intake without altering the corticosterone levels through undefined compensatory mechanisms. Importantly, administration of CRH by i.c.v. injection reduced food intake during 2 h to the indistinguishable level between $Agtr1a^{-/-}$ and $Agtr1a^{+/+}$ mice (Fig. 6). These results strongly suggest that down-regulation of anorexigenic *Crh* is the underlying mechanism of hyperphagia in $Agtr1a^{-/-}$.

AT_1 Receptor Signaling Regulates Crh Gene Transcription via cAMP-responsive Element—Next, to examine how AT_1 receptor signaling regulates *Crh* gene transcription, we transfected a luciferase reporter containing the −907 bp promoter of the human *Crh* gene (−907 Crh-Luc) into HEK293 cells expressing AT_1 receptor. After stimulation with 10^{-7} M of Ang II, a strong transactivation of the *Crh* gene promoter was observed (Fig. 7*A*). Deletion of the *Crh* promoter between −907 and −220 markedly reduced the fold activation by Ang II stimulation, although deletion between −907 and −330 had a marginal effect (Fig. 7*A*). Deletion of the cAMP-responsive element (CRE) located between −330 and −220 of the *Crh* promoter (14, 25) significantly reduced the fold activation by Ang II stimulation (Fig. 7*B*), and co-transfection of an expression vector for DN-CREB diminished Ang II-induced fold activation of −907 Crh-Luc in a dose-dependent manner (Fig. 7*C*). These results suggest that the AT_1 receptor signaling regulates *Crh* gene transcription via CRE.

DISCUSSION

Our present study demonstrated a hitherto undefined role of the AT_1 receptor signaling in the regulation of hypothalamic neurocircuit that is involved in the control of food intake. Ang II is produced by cleavage of angiotensiongen by renin and ACE. Mice deficient for angiotensinogen (*Agt*) or renin (*Ren1c*) are similarly resistant to diet-induced weight gain (26, 27). On a high-fat diet, no differences in food intake were observed between these mutant mice and wild-type mice, although the food intake of $Agt^{-/-}$ mice was significantly more than that of $Agt^{+/+}$ mice on a standard chow diet (26). On the other hand, mice deficient for ACE (*Ace*) are lean, exhibiting unchanged food intake and increased energy expenditure (28). Insomuch

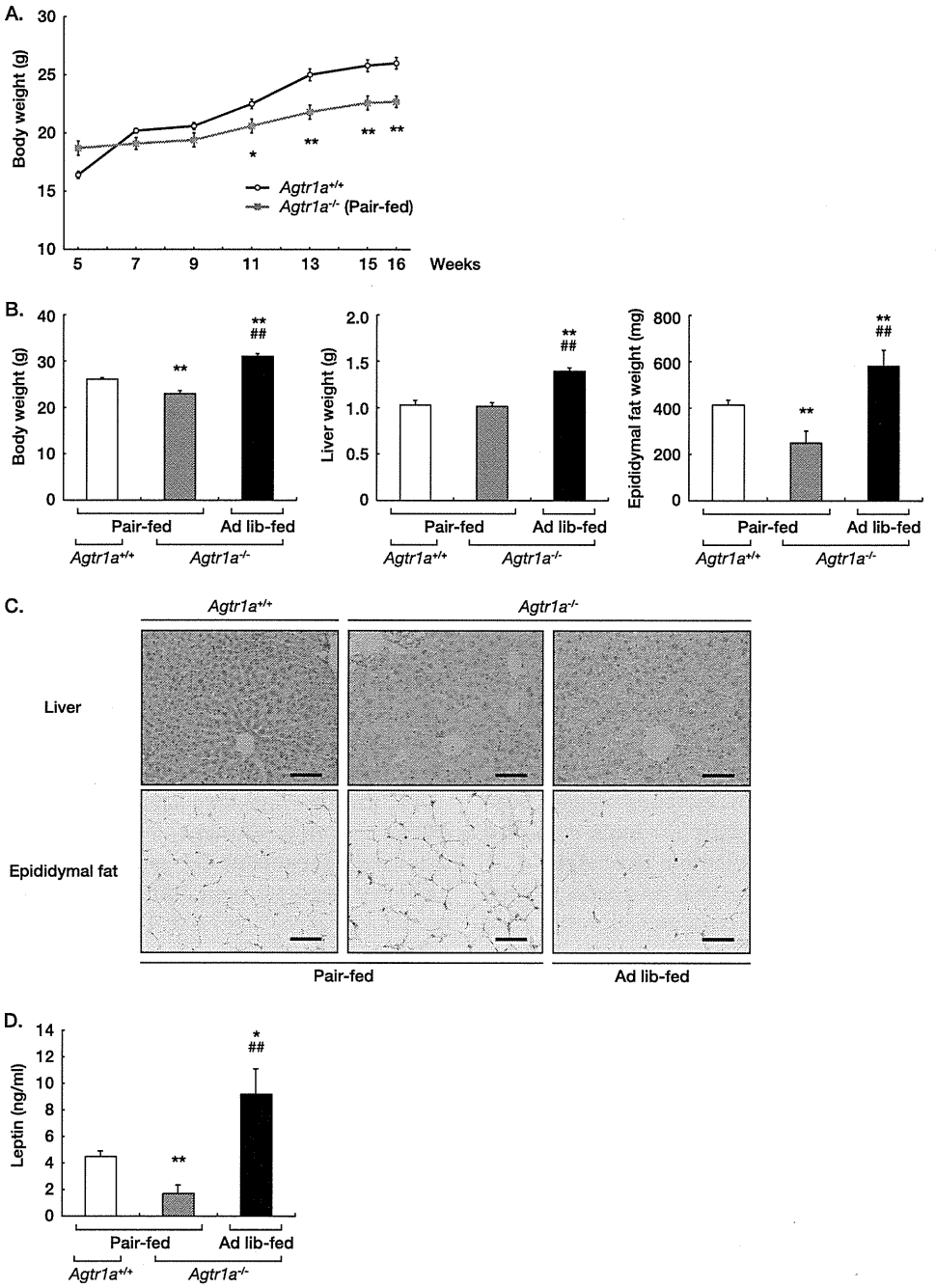

FIGURE 4. **Pair feeding supernormalizes obesity in *Agtr1a*$^{-/-}$ mice.** *A*, growth curves of male *Agtr1a*$^{+/+}$ ($n = 13$) and pair-fed *Agtr1a*$^{-/-}$ ($n = 13$) mice. Male *Agtr1a*$^{-/-}$ mice were pair-fed with the same amount of chow consumed by *Agtr1a*$^{+/+}$ mice from 5 to 16 weeks of age. *, $p < 0.05$; **, $p < 0.01$. *B*, body and organ weight of male *Agtr1a*$^{+/+}$ ($n = 10$), pair-fed *Agtr1a*$^{-/-}$ ($n = 7$), and *ad libitum* (*Ad lib*)-fed *Agtr1a*$^{-/-}$ ($n = 10$) mice at 16 weeks of age. *, $p < 0.05$ *versus* *Agtr1a*$^{+/+}$; **, $p < 0.01$ *versus* *Agtr1a*$^{+/+}$; #, $p < 0.05$ *versus* pair-fed *Agtr1a*$^{-/-}$; ##, $p < 0.01$ *versus* pair-fed *Agtr1a*$^{-/-}$. *C*, histological analysis of liver and epididymal fat of male *Agtr1a*$^{+/+}$, pair-fed *Agtr1a*$^{-/-}$, and *ad libitum*-fed *Agtr1a*$^{-/-}$ mice at 16 weeks of age. All sections were stained with hematoxylin and eosin. *Scale bars*, 50 μm. *D*, serum leptin concentrations in male *Agtr1a*$^{+/+}$ ($n = 7$), pair-fed *Agtr1a*$^{-/-}$ ($n = 7$), and *ad libitum*-fed *Agtr1a*$^{-/-}$ ($n = 7$) mice at 16 weeks of age. *, $p < 0.05$ *versus* *Agtr1a*$^{+/+}$; **, $p < 0.01$ *versus* *Agtr1a*$^{+/+}$; #, $p < 0.05$ *versus* pair-fed *Agtr1a*$^{-/-}$; ##, $p < 0.01$ *versus* pair-fed *Agtr1a*$^{-/-}$.

TABLE 1

Urinary excretions of catecholamines in pair-fed *Agtr1a*$^{+/+}$ ($n = 7$) and *Agtr1a*$^{-/-}$ ($n = 7$) mice at 6 weeks of age

	Agtr1a$^{+/+}$	*Agtr1a*$^{-/-}$
Urine volume (μl/day)	632.0 ± 113.9	1836.1 ± 253.3[a]
Urinary norepinephrine (ng/day)	1.1 ± 0.2	5.9 ± 1.3[b]
Urinary epinephrine (ng/day)	8.5 ± 2.9	78.0 ± 20.6[b]
Urinary dopamine (ng/day)	92.4 ± 9.1	345.5 ± 93.6[b]

[a] $p < 0.01$.
[b] $p < 0.05$.

as the renal abnormalities observed in *Agt*$^{-/-}$ mice are quantitatively similar to those of mutant mice homozygous for both AT_{1a} and AT_{1b} (29, 30), it is possible that the effect of Ang II on AT_{1b} receptor might contribute to feeding behavior in *Agtr1a*$^{-/-}$ mice. However, the compensatory effect of Ang II receptor subtypes was minimal, because the mRNA levels of the AT_{1b} (*Agtr1b*) and AT_2 (*Agtr2*) receptors in the hypothalamus did not differ significantly between *Agtr1a*$^{+/+}$ and *Agtr1a*$^{-/-}$

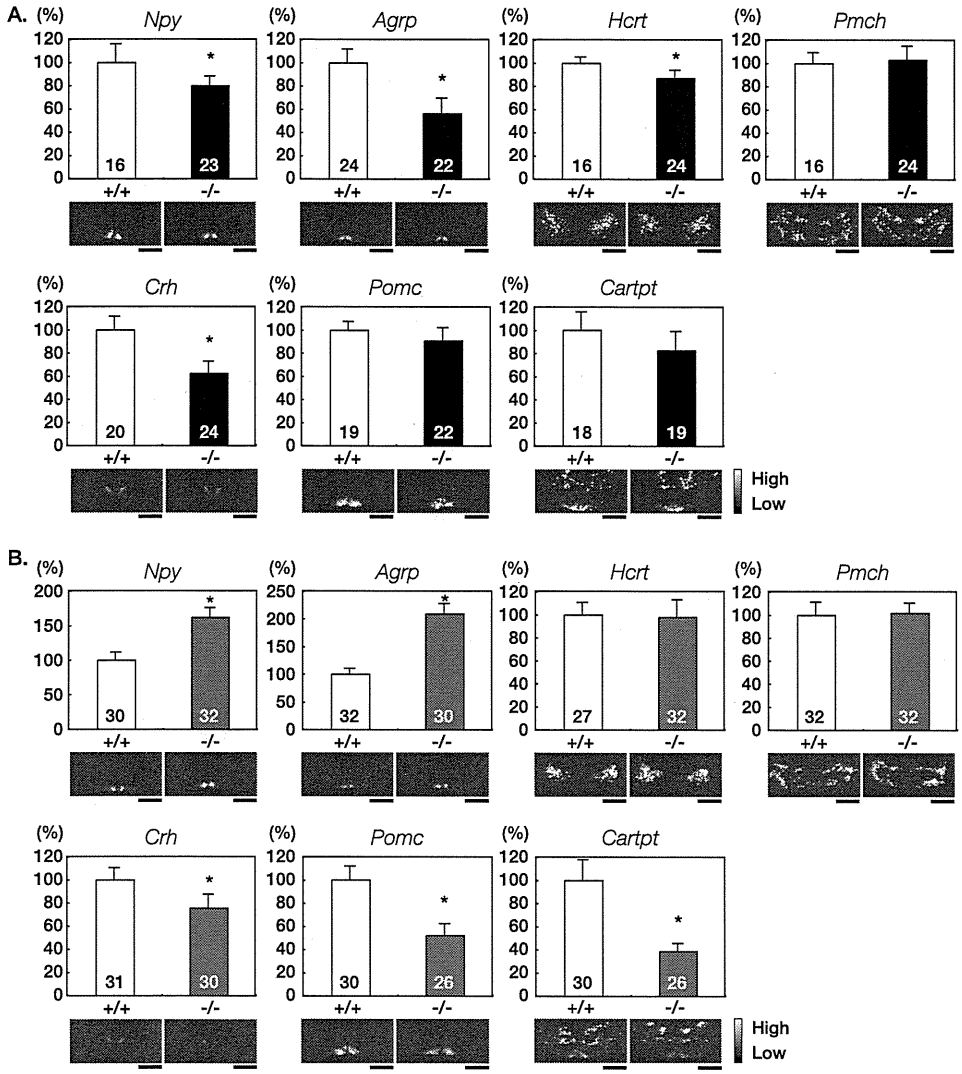

FIGURE 5. **Hypothalamic *Crh* expression is decreased in *ad libitum*- and pair-fed *Agtr1a*$^{-/-}$ mice.** *A*, quantitative *in situ* hybridization analysis of hypothalamic orexigenic and anorexigenic peptides in *ad libitum*-fed *Agtr1a*$^{+/+}$ and *Agtr1a*$^{-/-}$ mice at 16 weeks of age. The number of sections for each analysis is indicated in the *bars*. *, $p < 0.05$. *Scale bars*, 1 mm. *B*, quantitative *in situ* hybridization analysis of hypothalamic orexigenic and anorexigenic peptides in pair-fed *Agtr1a*$^{+/+}$ and *Agtr1a*$^{-/-}$ mice at 16 weeks of age. The number of sections for each analysis is indicated in the *bars*. *, $p < 0.05$. *Scale bars*, 1 mm.

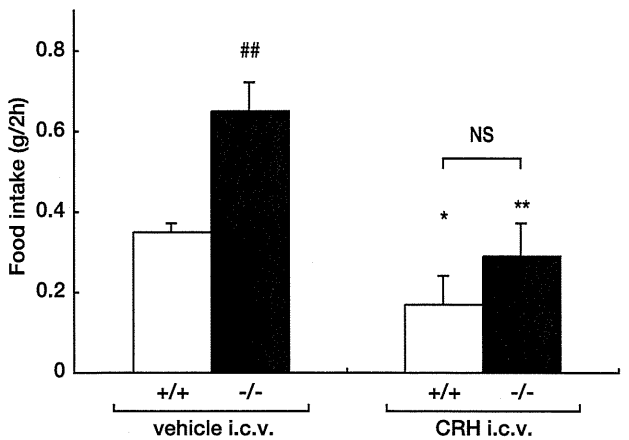

FIGURE 6. **i.c.v. injection of CRH reduces food intake in *Agtr1a*$^{-/-}$ mice.** Food intake over 2 h after i.c.v. administration of CRH or vehicle in *Agtr1a*$^{+/+}$ and *Agtr1a*$^{-/-}$ mice at 8 weeks of age. *, $p < 0.05$ *versus* vehicle; **, $p < 0.01$ *versus* vehicle; ##, $p < 0.01$ *versus* *Agtr1a*$^{+/+}$. *NS*, not significant.

mice (supplemental Fig. S2). Kouyama and colleagues reported that *Agtr1a*$^{-/-}$ mice showed attenuation of high-fat diet-induced weight gain and adiposity, which was accompanied by unchanged food intake and increased energy expenditure (15). However, they also described that *Agtr1a*$^{-/-}$ mice ate more on a calorie basis than *Agtr1a*$^{+/+}$ mice on a standard chow diet (15). In our hands, *Agtr1a*$^{-/-}$ mice were initially hyperphagic, compared with *Agtr1a*$^{+/+}$ mice, when maintained *ad libitum* on a high-fat diet (supplemental Fig. S3*A*). However, the difference in daily food intake between *Agtr1a*$^{-/-}$ and *Agtr1a*$^{+/+}$ mice became insignificant after 6 weeks of high-fat diet (supplemental Fig. S3*B*). The reasons for this phenomenon are currently unclear, but unpalatable taste of high-fat chow and altered metabolic status due to high-fat diet might potentially contribute to it. As a consequence, *Agtr1a*$^{-/-}$ weighed 9.8 ± 3.7% more than *Agtr1a*$^{+/+}$ mice after 6 weeks of high-fat diet (supplemental Fig. S3*B*), but the difference in body weight between *Agtr1a*$^{-/-}$ and *Agtr1a*$^{+/+}$ mice was not statistically significant ($p = 0.053$). We assume that to gain or to

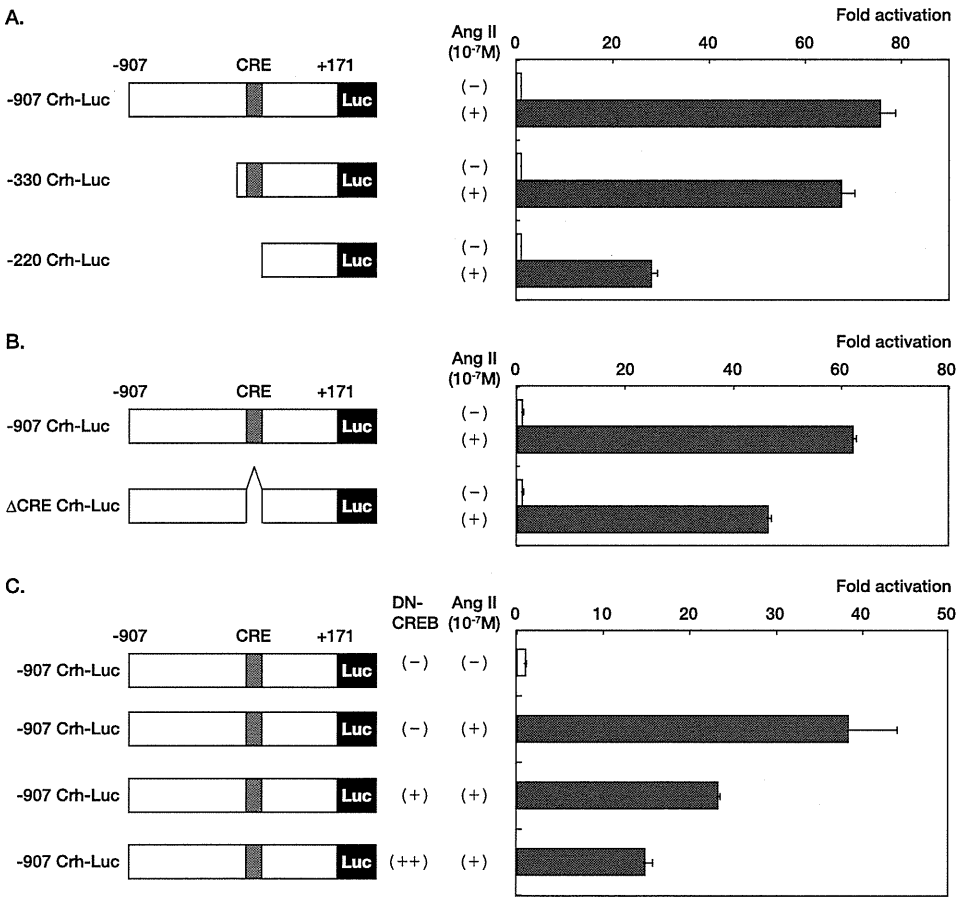

FIGURE 7. **AT_1 receptor signaling regulates *Crh* gene transcription.** *A* and *B*, the luciferase reporters containing full-length (−907 Crh-Luc), deleted (−330 Crh-Luc or −220 Crh-Luc), or CRE-mutated (ΔCRE Crh-Luc) were transfected in HEK293 cells expressing AT_1 receptor (HEK293-AT_1 cells). The cells were stimulated with Ang II (10^{-7} M) 24 h before the measurement of luciferase activities. *C*, the luciferase reporters containing full-length *Crh* promoter (−907 Crh-Luc) was co-transfected in HEK293-AT_1 cells with an expression plasmid of DN-CREB. The cells were stimulated with Ang II (10^{-7} M) 24 h before the measurement of luciferase activities.

lose weight may be determined upon a delicate balance between the counteracting effects of the central *versus* peripheral function of AT_1 receptor signaling in *Agtr1a*$^{-/-}$ mice. Our *Agtr1a*$^{-/-}$ mice seem to be more susceptible to body weight gain than those used by Kouyama *et al.* (15), on a standard or high-fat chow, which might be caused by the differences in the extent of energy expenditure, potentially resulting from different housing conditions. Further studies will be needed to elucidate the differential regulation of feeding behavior by RAS components.

It has been reported that neuroendocrine dysfunction including CRH hyperactivity has been associated with anorexia nervosa (31), and AT_1 receptor expression in the PVN is increased in several stress models, contributing to enhanced CRH production and release (32). Although AT_1 receptor blockers are widely used to treat hypertension, the effects of AT_1 receptor blockers on feeding behavior have not been well documented in clinical practice. Subcutaneous administration of an AT_1 receptor blocker olmesartan in wild-type mice lowered blood pressure to the level comparable with that of *Agtr1a*$^{-/-}$ mice (supplemental Fig. S4*A*), but the body weight and daily food intake did not differ significantly between olmesartan- and vehicle-treated mice (supplemental Fig. S4*B*). In addition, the expression levels of hypothalamic appetite-related peptides genes were unchanged (supplemental Fig. S4*C*). On the other hand, i.c.v. administration of olmesartan significantly increased food intake in wild-type mice (supplemental Fig. S5). These data indicate that peripheral administration of olmesartan is insufficient to inhibit the AT_1 receptor signaling in the PVN and thus has little effect on hypothalamic neurocircuits and food intake. However, we propose that the hypothalamic AT_1 receptor signaling will be a therapeutic target against neurotic and stress-induced eating disorders, if we develop an AT_1 receptor blocker with high ability to penetrate the PVN nuclei and to inhibit the hypothalamic AT_1 receptor signaling effectively.

We conclude that the AT_1 receptor signaling is involved in feeding behavior by regulating *Crh* gene expression in the PVN. Elucidation of the mechanism by which the AT_1 receptor signaling regulates feeding behavior via neuropeptides circuits will provide a clue to the management of cardiovascular, metabolic, and eating disorders.

Acknowledgments—We thank A. Furuyama, M. Ikeda, Y. Ohtsuki, I. Sakamoto, and M. Kikuchi for excellent technical assistance. We thank Dr. M. Nakazato (Miyazaki University) for valuable comments and critical reading of our manuscript.

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Official Journal of the Japanese Circulation Society
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Coronary Artery Bypass Grafting in Hemodialysis-Dependent Patients

– Analysis of Japan Adult Cardiovascular Surgery Database –

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Background: Perioperative risk during coronary artery bypass grafting (CABG) is reportedly high in patients with chronic renal disease. We aimed to determine postoperative mortality and morbidity and identify the perioperative risk factors of mortality during CABG in hemodialysis (HD)-dependent patients.

Methods and Results: From the Japan Adult Cardiovascular Surgery Database, we compared 1,300 HD-dependent chronic renal failure patients with 18,387 non-HD patients who all underwent isolated CABG between January 2005 and December 2008. The operative mortality and mortality, including major morbidity, was 4.8% vs. 1.4% and 23.1% vs. 13.7% in the HD and non-HD groups, respectively. Preoperative predictors of operative mortality included age, chronic obstructive pulmonary disease, peripheral arterial disease, congestive heart failure, arrhythmia, preoperative inotropic agent requirement, New York Heart Association class IV, urgent or emergency operation, poor left ventricular function, aortic valve regurgitation (>2), and mitral valve regurgitation (>3). Postoperative predictors of operative mortality included stroke, infection, prolonged ventilation, pneumonia, heart block, and gastrointestinal complications.

Conclusions: Compared with non-HD patients, CABG in HD patients was associated with high mortality and morbidity rates. An appropriate surgical strategy and careful perioperative assessment and management for prevention of respiratory and gastrointestinal complications might contribute to improved clinical outcomes after CABG in these patients.

Key Words: Coronary artery bypass grafting; Hemodialysis; Risk factor

Coronary artery disease (CAD) frequently occurs in patients with chronic renal failure (CRF) and is a major cause of mortality and morbidity in these patients.[1] CRF patients with CAD often need myocardial revascularization, and of the revascularization techniques, coronary artery bypass grafting (CABG) has been reported as having satisfactory survival rates in patients with kidney disease.[2,3] CABG has also been shown to yield better overall and angina-free survival than does percutaneous coronary intervention (PCI).[4–6] However, the operative mortality and morbidity are reportedly high compared with those of non-hemodialysis (HD) patients in both the short- and long-term. The hospital death rate after isolated CABG in HD-dependent patients was reported to be approximately 10%, which was higher than that for PCI.[7,8]

Editorial p???

Therefore, an appropriate surgical strategy and perioperative medical treatment based on the identification of perioperative risk factors would lead to an improvement in the clinical outcomes of these surgical procedures. For the past few decades, various studies have reported the clinical outcome of cardiac surgery in CRF patients, but almost all have been from single centers or consist of less than 200 patients.[1] Moreover, we found few previous large-scale studies that focused on isolated CABG and included multivariate analysis of perioperative risk factors of operative mortality.[8]

Therefore, in the present study we examined 19,687 isolated CABG patients, including 1,300 HD-dependent patients,

Received October 10, 2011; revised manuscript received December 27, 2011; accepted January 6, 2012; released online February 14, 2012
Time for primary review: 23 days

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ISSN-1346-9843 doi:10.1253/circj.CJ-11-1146

Table 1. Baseline Characteristics of Patients Undergoing CABG

	Non-HD (n=18,387)	HD (n=1,300)	P value
Age, years			
≤60	17.9	28.3	<0.0001
61–65	13.8	19.0	
66–70	18.6	19.3	
71–75	22.0	17.9	
76–80	18.7	11.6	
≥81	9.0	3.8	
Mean age, years	68.7±9.4	65.4±9.2	<0.0001
BSA	1.64±0.39	1.59±0.16	0.001
Male (%)	77.3	78.7	0.246
History of smoking (%)	53.0	46.2	<0.0001
Current smoking (%)	20.0	16.7	0.004
DM (%)	48.0	65.7	<0.0001
DM requiring medication (%)	40.7	56.9	<0.0001
Serum creatinine (mg/dl)	1.01±1.1	8.61±25.2	<0.0001
Hyperlipidemia (%)	57.0	36.5	<0.0001
Hypertension (%)	73.3	83.1	<0.0001
History of cerebrovascular event (%)	13.5	17.7	<0.0001
Recent (<2 weeks) cerebrovascular event (%)	0.6	0.8	0.295
History of infective endocarditis (%)	0.1	0.2	0.342
Chronic lung disease (moderate, severe) (%)	1.5	1.4	0.650
Extracardiac arterial disease (%)	15.4	27.0	<0.0001
Peripheral arterial disease (%)	14.3	25.4	<0.0001
Thoracic aortic disease (%)	2.1	2.2	0.699
Mental disorder (%)	3.1	4.5	0.006
History of coronary intervention (%)	25.1	28.1	0.015
Previous MI (%)	34.7	31.1	0.008
Congestive heart failure (2 weeks) (%)	13.7	22.8	<0.0001
Angina (%)	88.2	89.9	0.056
Unstable (%)	28.8	34.7	<0.0001
Cardiogenic shock (%)	4.3	6.6	0.0001
Arrhythmia (%)	7.4	9.7	0.003
Inotropic agents requirement (%)	3.5	4.9	0.010
Reoperation (%)	2.2	2.3	0.875
Urgent operation (%)	11.8	13.7	0.044
Emergency operation (%)	6.9	8.7	0.017
BMI mean	24.0±29.7	22.7±14.0	0.003
>26 (%)	21.5	12.0	<0.0001
>30 (%)	3.7	2.2	0.004
NYHA class			
NA (%)	14.1	10.1	<0.0001
I (%)	26.3	23.1	0.011
II (%)	36.2	35.2	0.465
III (%)	14.1	17.8	0.0003
III or IV (%)	23.0	30.8	<0.0001
No. of diseased vessels (%)			
1	4.4	4.9	0.381
2	24.8	23.9	0.465
3	69.4	70.2	0.531
Ejection fraction (%)			
>60	48.9	34.1	<0.0001
30–60	44.4	55.4	<0.0001
<30	6.2	9.8	<0.0001
AS (%)	1.7	6.5	<0.0001
MS (%)	0.3	1.2	<0.0001
AVR (>2) (%)	5.6	5.7	0.845
MVR2 (>2) (%)	11.5	20.7	<0.0001
TVR2 (>2) (%)	5.4	8.7	<0.0001
AVR3 (>3) (%)	0.6	0.7	0.768
MVR3 (>3) (%)	1.4	4.4	<0.0001
TVR3 (>3) (%)	0.6	1.3	0.002

CABG, coronary artery bypass grafting; HD, hemodialysis; BSA, body surface area; DM, diabetes mellitus; MI, myocardial infarction; BMI, body mass index; NYHA, New York Heart Association; AS, aortic stenosis; MS, mitral stenosis; AVR, aortic valve regurgitation; MVR, mitral valve regurgitation; TVR; tricuspid valve regurgitation.

from between 2005 and 2008 in the Japan Adult Cardiovascular Surgery Database (JACVSD) to determine the contemporary clinical outcome of isolated CABG and to determine the risks for perioperative death following CABG in patients with HD-dependent CRF. We then discuss the appropriate surgical strategy for and the perioperative medical management of such patients.

Methods

Study Population

The JACVSD was initiated in 2000 to estimate surgical outcomes after cardiovascular procedures in many centers throughout Japan. The JACVSD adult cardiovascular division currently captures clinical information from nearly half of all Japanese hospitals performing cardiovascular surgery. The data collection form has a total of 255 variables (definitions are available online at http://www.jacvsd.umin.jp), and these are almost identical to those in the Society of Thoracic Surgeons (STS) National Database (definitions are available online at http://sts.org). The JACVSD has developed software for a web-based data collection system through which the data manager of each participating hospital electronically submits the data to the central office. Although participation in the JACVSD is voluntary, data completeness is a high priority. Accuracy of submitted data is maintained by data audit achieved by monthly visits by administrative office members to the participating hospital to check data against clinical records. Validity of data is further confirmed by an independent comparison of the volume of cardiac surgery at a particular hospital entered in the JACVSD with that reported to the Japanese Association for Thoracic Surgery annual survey.[9]

We examined cases of isolated CABG between January 1, 2005 and December 31, 2008. JACVSD records that had been obtained without the patient's informed consent were excluded from this analysis. Records with missing or out of range age, sex, or 30-day status (see Endpoints section below) were also excluded. After data cleaning, the population for this risk model analysis consisted of 1,300 HD-dependent patients and 18,387 non-HD-dependent patients who underwent cardiovascular procedures at 167 participating sites throughout Japan.

Endpoints

The primary outcome measure of the JACVSD was 30-day operative mortality, which was defined exactly the same as the 30-day operative mortality in the STS National Database. The 30-day operative mortality included any patient who died during the index hospitalization, regardless of the length of hospital stay, and any patient who died after being discharged from hospital within 30 days of the operation. Operative mortality also included any patients who died after 31 days during the hospital stay in addition to patients included in the 30-day mortality. Using a definition from previous studies,[10,11] major morbidity was defined as any of the following 5 postoperative in-hospital complications: stroke, reoperation for any reason, need for mechanical ventilation for more than 24h after surgery, renal failure, or deep sternal wound infection.

Statistical Analysis

We examined differences between 2 groups (isolated CABG with and without HD) using bivariate tests: Fisher's exact test and the chi-square test for categorical covariates, and the unpaired t-test or Wilcoxon rank sum test for continuous covariates. To develop risk models of isolated CABG with HD, we conducted multivariate stepwise logistic regression analysis for each outcome. Stability of the model was checked every time a variable was eliminated. When all statistically non-significant variables (P<0.10) had been eliminated from the model, "goodness-of-fit" was evaluated and the area under the receiver-operating characteristic curve was used to assess how well the model could discriminate between patients who lived from those who had died. To investigate the relationship between postoperative complications and operative death in HD patients, we conducted multivariate stepwise logistic regression analysis for operative mortality. Complications such as cardiac arrest and multisystem failure were excluded from this analysis because they are highly associated with operative death.

Results

Patient Demographics

Baseline characteristics of the study population are summarized in **Table 1**. Patients in the HD group were significantly younger (65.4±9.2 vs. 68.7±9.4 years) and had less body surface area (1.59 vs. 1.64m^2) than the non-HD patients. As expected, the HD-dependent patients had a significantly greater degree of baseline comorbidity than did non-HD patients. Patients in the HD group were more likely to have a history of diabetes (56.9% vs. 40.7%), hypertension (83.1% vs. 73.3%), and peripheral vascular disease (27.0% vs. 15.4%). A higher rate of current congestive heart failure (22.8% vs. 13.7%) with a lower ejection fraction and lower New York Heart Association (NYHA) status was observed in the HD group. As for valvular disease, aortic stenosis (6.5% vs. 1.7%), mitral valve regurgitation (MVR) (>2) (20.7% vs. 11.5 %), and tricuspid valve regurgitation (>2) (8.7% vs. 5.4%) were more common in HD patients. In both the HD and non-HD groups, off-pump surgery was performed approximately twice as often as on-pump surgery, and the off-pump ratio did not differ between groups. Transfusion was required more often in the HD group. Bilateral internal mammary artery usage in the HD group was less frequent than in the non-HD group (22.8% vs. 31.4%) (**Table 2**).

Postoperative Outcomes

In-hospital outcomes are summarized in **Table 3**. The 30-day mortality was 4.8% vs. 1.4% and the operative mortality was 7.8% vs. 2.1% in the HD and non-HD groups, respectively. Both the 30-day and operative mortalities in HD patients were approximately 3-fold more frequent than in non-HD patients. Operative mortality with a major complication was more frequent in the HD group (23.1% vs. 13.7%).

Table 2. Intraoperative Characteristics

	Non-HD CABG (n=18,387)	HD CABG (n=1,300)	P value
On-pump surgery (%)	37.3	35.6	0.230
Transfusion (%)	52.5	87.8	<0.0001
Bilateral IMA usage (%)	31.4	22.8	<0.0001
Single IMA usage (%)	61.7	68.7	<0.0001

IMA, internal mammary artery. Other abbreviations see in Table 1.

Table 3. Mortality and Morbidity

	Non-HD CABG (n=18,387)	HD CABG (n=1,300)	P value
30-day mortality (%)	1.4	4.8	<0.0001
Operative mortality (%)	2.1	7.8	<0.0001
Operative mortality+major complication (%)	13.7	23.1	<0.0001
Reoperation (any reason)	5.4	6.6	0.067
Infection			
Deep sternum	1.8	2.6	0.026
Thoracotomy	0.5	1.1	0.007
Leg	1.9	4.5	<0.0001
Urinary	0.8	0.6	0.402
Septicemia	1.0	2.7	<0.0001
Prolonged ventilation	6.6	9.4	<0.0001
Pneumonia	2.4	4.4	<0.0001
Pulmonary embolism	0.2	0.2	0.989
Stroke	1.5	1.6	0.636
TIA	1.3	2.4	0.001
Coma	0.6	1.1	0.017
Paraparesis	0.3	0.4	0.871
Atrial fibrillation	13.2	14.9	0.076
Heart block requiring pacemaker	0.5	0.8	0.159
Cardiac arrest	1.0	2.8	<0.0001
Reoperation for bleeding	1.8	3.0	0.003
Anticoagulant complication	0.3	0.5	0.084
Tamponade requiring drainage	1.0	1.4	0.132
Gastrointestinal complication	1.6	3.9	<0.0001
Multisystem failure	0.9	2.6	<0.0001
Dissection aorta	0.1	0	0.378
Dissection iliac	0.02	0.1	0.138
Limb ischemia	0.2	0.7	0.001
Re-admission	1.9	2.5	0.193
ICU stay >8 days	5.7	11.2	<0.0001

TIA, transient ischemic attack; ICU, intensive care unit. Other abbreviations see in Table 1.

Multivariate Predictors of In-Hospital Death

Multivariate predictors of operative mortality are summarized in **Table 4**. Predictors of operative mortality included

Table 4. Multivariate Preoperative Predictors of Operative Mortality of CABG for HD Patients

Characteristic	RR (95%CI)	P value
Age	1.38 (1.184–1.604)	<0.0001
Chronic pulmonary disease (moderate severe)	5.52 (1.786–17.033)	0.003
Extracardiac arterial disease	1.86 (1.15–3.01)	0.011
Congestive heart failure	1.77 (1.06–2.957)	0.029
Arrhythmia	1.84 (1.02–3.325)	0.043
Preoperative inotropic agent	2.46 (1.204–5.024)	0.014
NYHA class IV	1.99 (1.1–3.599)	0.023
Urgent operation	2.02 (1.085–3.752)	0.027
Emergency operation	2.27 (1.177–4.372)	0.014
Ejection fraction <30%	2.06 (1.125–3.787)	0.019
AVR ≥2	3.98 (1.987–7.979)	<0.0001
MVR ≥3	2.32 (1.094–4.913)	0.028

RR, relative risk; CI, confidence interval. Other abbreviations see in Table 1.

Table 5. Multivariate Postoperative Predictors of Operative Mortality of CABG for HD Patients

	OR	CI	P value
Stroke	9.85	3.1–30.8	<0.0001
Infection	6.72	2.6–17.7	<0.0001
Prolonged ventilation	3.82	2.1–7.0	<0.0001
Pneumonia	13.15	6.3–27.4	<0.0001
Gastrointestinal complication	5.43	2.3–12.7	<0.0001
Heart block	12.46	2.4–64	0.003

OR, odds ratio. Other abbreviations see in Tables 1,4.

age (odds ratio [OR]=1.38, P<0.0001), chronic obstructive pulmonary disease (COPD) (OR=5.52, P=0.003), peripheral arterial disease (OR=1.86, P=0.011), congestive heart failure (OR=1.77, P=0.029), arrhythmia (OR=1.84, P=0.043), preoperative inotropic agent requirement (OR=2.46, P=0.014), NYHA class IV (OR=1.99, P=0.023), urgent operation (OR=2.02, P=0.027), emergency operation (OR=2.27, P=0.014), ejection fraction <30% (OR=2.06, P=0.019), aortic valve regurgitation (AVR) (>2) (OR=3.98, P<0.0001), and MVR (>3) (OR=2.32, P=0.028).

Relationship Between Operative Mortality and Postoperative Complications

Results are summarized in **Table 5**. Among the complications observed relatively often (incidence >3%), prolonged ventilation (OR=3.82), pneumonia (OR=13.15), infection (OR=6.72), and gastrointestinal complications (OR=5.43) were significant factors in operative mortality.

Discussion

We investigated the clinical outcomes and risk factors of operative mortality and morbidity in patients with (n=1,300) and without (n=18,387) HD who underwent isolated CABG. The study data was extracted from the JACVSD, and is one of the largest comparative series of post-CABG outcomes in such patients.[8,12]

The operative mortality of HD patients after isolated CABG in this study was 7.8%, which was similar to previous studies that reported an operative mortality of approximately 10%.[8,12] As previously reported, HD patients have more preoperative comorbidities. Compared with other reports, the rates of emergency operation, male sex, shock state, and off-pump CABG tended to be high in this study, and those of congestive heart failure and chronic lung disease tended to be low. Age, hypertension, NYHA status, and prevalence of valvular disease were comparable. The postoperative morbidity rate of the HD group was higher than that in the non-HD group. Major postoperative morbidity (stroke, prolonged ventilation, deep sternal infection, renal failure and reoperation for any reason) were also comparable with those in the reports from the STS database, which included 7,152 dialysis patients.[8] Besides the major complications, the prevalence of leg infection, pneumonia, transient ischemic attack, cardiac arrest, gastrointestinal complications, multisystem failure, and limb ischemia in HD patients was significantly higher than in non-HD patients.

A series of studies have reported early and late outcomes of CABG with and without valve operations in CRF patients.[13–16] In those studies, several risk factors were reported for mortality after cardiac surgery in HD-dependent patients. Many reports have found a low ejection fraction to be an independent risk factor,[17–20] which was consistent with the findings of the present study. However, we found no previous large-scale studies that focused on isolated CABG and included a multivariate analysis of risk factors for hospital mortality. As a large-scale report that focused on isolated CABG, Cooper et al demonstrated that the glomerular filtration rate was a powerful predictor of operative morbidity after isolated CABG in 7,152 HD patients.[8] Charytan et al analyzed 77,323 non-HD and 635 HD patients who underwent CABG that included valve surgery. They demonstrated that HD-dependence, congestive heart failure, valvular heart disease, valve surgery, female sex, age, pathological weight loss, chronic lung disease, neurological disorders, admission for myocardial infarction, and liver disease were adjusted risks for perioperative mortality.[12]

Regarding valvular disease, we also demonstrated that AVR (>2) and MVR (>3) were independent risk factors for operative mortality after isolated CABG. The question then arose regarding whether valve operation should be performed simultaneously with CABG when moderate AVR or MVR was complicated. Horst et al reported that the risk for perioperative death associated with CABG combined with valve operation was approximately 10-fold that for isolated CABG.[1] Charytan et al also demonstrated concomitant valve surgery as a perioperative risk factor.[12] the surgical management of moderate, chronic ischemic MVR combined with CABG is still controversial.[21,22] Combined mitral valve surgery has been reported to be significantly associated with a lower residual grade of MVR compared with CABG alone. On the other hand, it has been reported that CABG alone was able to reduce the MVR grade in 40% of patients.[23] From the postoperative NYHA status perspective, the effect of mitral valve surgery is also controversial.[23,24] As for late mortality, a meta-analysis of 2,479 ischemic MVR patients showed that mitral valve surgery did not have advantages for late mortality compared with CABG alone.[24] As for aortic valve disease, most surgeons would not perform concomitant aortic valve surgery in HD patients with AVR=2. However, concomitant aortic valve surgery might be taken into consideration in some cases complicated by AVR >3. In the present study, the cohort of AVR >3 was very small (non-HD group: <100 patients, HD group: <10 patients.) Therefore, it was very difficult to investigate whether AVR >3 is a risk factor or not for postoperative mortality in our multivariate analysis.

In summary, concomitant surgery should be performed with consideration of the "risks and benefits" of additional valve surgery, and further study is necessary.

Relatively few large-series reports have documented the detailed incidence of postoperative morbidity after isolated CABG in HD-dependent patients.[8,12] Compared with non-HD patients, the incidence of major postoperative complications was high in HD-dependent patients in the present study, as previously reported. Postoperative complications could be considered to be closely associated with higher mortality. The higher incidence of these complications in HD-dependent patients might partly explain their poor clinical outcomes. To improve the clinical outcome of isolated CABG in HD, it seems important to prevent these complications. The complications that occurred at a relatively high incidence (>3%) in the present study were infection, prolonged ventilation, pneumonia, atrial fibrillation, reoperation for bleeding, and gastrointestinal complications. Among these, infection, prolonged ventilation, pneumonia, and gastrointestinal complications were significant independent risk factors for operative mortality.

Several studies have reported that higher mortality rates are associated with infection in HD-dependent patients undergoing cardiac surgery. Takami et al reported that following cardiac surgery in their 245 HD patients, almost half of the cases of hospital death were related to infection.[25] Akman et al demonstrated that infection was an independent postoperative risk factor for mortality after CABG in HD-dependent patients, and suggested the importance of early diagnosis of infection for both early recovery and shorter hospitalization in the postoperative period.[7]

Mangi et al reported that the incidence of gastrointestinal complications requiring surgical repair after cardiac and vascular surgery was 0.53% (46/8,709).[26] Of these, mesenteric ischemia comprised 67% and two-thirds of these patients died. To prevent gastrointestinal complications, especially mesenteric ischemia, preoperative abdominal screening, bowel preparations, and postoperative volume control might be important in isolated CABG for HD-dependent patients.

To prevent postoperative pulmonary complications, preoperative risk stratification and a risk-reduction strategy seem important. As for postoperative respiratory complications, COPD, the prevalence of which is higher by 10–12% among cardiovascular surgical candidates compared with aged-matched populations, might be one of the most important risk factors.[27] Several advances in surgery and anesthetic care have been shown to be particularly beneficial for COPD patients. Compared with standard operations, minimally invasive procedures produce less tissue damage and, in turn, attenuated neurohumoral and inflammatory responses.[27] Off-pump bypass is considered to be a minimally invasive surgery in coronary artery operations. Patients with FEV_1 (1 s to forced vital capacity) less than the lower limit of normal have better outcomes after off-pump bypass compared with those post-CABG.[28]

Conclusions

Compared with non-HD patients, CABG in HD patients was associated with high mortality and morbidity rates in the present study. An appropriate surgical strategy and careful perioperative assessment and management for prevention of respiratory and gastrointestinal complications might contribute to improvements in clinical outcomes after CABG in HD-dependent patients.

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