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Effects of Angiotensin Receptor Antagonist and Angiotensin Converting Enzyme Inhibitor on Insulin Sensitivity in Fructose-Fed Hypertensive Rats and Essential Hypertensives

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This study was designed to investigate the effects of angiotensin II (AII) receptor antagonist and angiotensin converting enzyme (ACE) inhibitor on insulin resistance, and the mechanism by which ACE inhibitor improves insulin-dependent glucose uptake (insulin sensitivity) in an insulin-resistant hypertensive rat model (fructose-fed rats, FFR) and in essential hypertensives (EHT).

Male Sprague-Dawley rats were fed on fructose-rich or standard chow for 4 weeks and treated either with 10 mg/kg/day of delapril (n = 8), 1 mg/kg/day of TCV-116 (AII receptor antagonist; n = 13), or vehicle (n = 9) for the latter 2 weeks. Steady-state plasma glucose (SSPG) was measured with the subjects in the conscious state; simultaneously, we infused insulin (2.5 mU/kg/min) and glucose (8 mg/kg/min) to determine insulin sensitivity in each group. Thirteen EHT were hospitalized and the 2-h euglycemic hyperinsulinemic glucose clamp (GC) method was performed in a fasting condition before and after 2 weeks' administration of TCV-116 (8 mg/day) in 7 EHT and of delapril (120 mg/day) in 6 EHT. Insulin sensitivity was evaluated as M-value calculated from the infusion rate of glucose.

Mean blood pressure (MBP) was higher in FFR (137.7 ± 73.8 mm Hg, $P < .05$) compared to controls (120.8 ± 2.7 mm Hg), and was lower in both the delapril (108.1 ± 6.3 mm Hg, $P < .05$) and TCV-116 (112.8 ± 4.3 mm Hg, $P < .05$) groups than in FFR. SSPG was higher in FFR (209.3 ± 7.6 mg/dL, $P < .01$) compared to controls (136.8 ± 10.1 mg/dL), and was lower in the delapril (170.8 ± 4.2 mg/dL, $P < .05$) and TCV-116 (171.7 ± 6.8 mg/dL, $P < .05$) groups. There was no significant difference between the delapril and TCV-116 groups in SSPG levels. In EHT, delapril and TCV-116 decreased MBP. M-value in the control period in EHT was lower than in normal controls in this study. After delapril and TCV-116 treatment, M-value was significantly higher to the same extent as that observed in the control period.

Thus, both ACE inhibitor and AII receptor antagonist improved insulin resistance as assessed by SSPG in FFR and by GC in EHT, suggesting that the improvement of insulin resistance by ACE inhibitor might depend on suppression of AII action.
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There is growing evidence that the renin-angiotensin system plays an important pathophysiologic role in cardiovascular diseases and clinical studies continue to show benefits from treatment of such patients with angiotensin converting enzyme (ACE) inhibitor. Recently, insulin resistance and the accompanying hyperinsulinemia have been reported to play an important role in the occurrence and maintenance of essential hypertension, dyslipidemia, and arteriosclerosis.[1–4] Several kinds of hypotensive drugs, such as thiazide diuretics and β-blockers, have been reported to impair insulin sensitivity.[5,6] On the other hand, it has been recognized that ACE inhibitor improves insulin sensitivity in essential hypertension,[5–8] suggesting an important role for the suppression of angiotensin II generation. ACE inhibitor also increases kinin activity, which has raised some doubts as to whether the insulin-sensitivity–improving effect of the ACE inhibitor is mediated entirely through the inhibition of angiotensin II generation. It has been reported that augmented kinin activity may contribute to the effect of ACE inhibitor in insulin sensitivity.[9,10] As the mechanism of ACE inhibitors which improves insulin sensitivity, it has been proposed that vasodilation increases delivery of glucose and insulin, that kinin has a direct effect on the glucose metabolism similar to insulin, and that norepinephrine release may be suppressed by lowered angiotensin II activity. If the ACE inhibitor augments insulin sensitivity through enhanced endogenous kinin, it would appear to be more effective for treating this condition than the angiotensin II receptor antagonist. Therefore, evaluation of the blocking effects on the angiotensin II receptor can help to elucidate the mechanism of ACE inhibitor in insulin sensitivity. However, it has not been clarified whether the angiotensin II receptor antagonist improves insulin sensitivity or not.

While the angiotensin II receptor has previously been blocked with peptide compounds such as saralasin, saralasin is not orally active. Also, it has a short duration of action, and has a partial agonistic action.[10] TCV-116 is a new specific angiotensin II receptor antagonist with no agonistic properties, and it provides the opportunity to study the consequences of blocking angiotensin II. It is well known that fructose feeding can cause blood pressure elevation, insulin resistance, and hyperinsulinemia in normal rats.[11,12] This study was designed to clarify the effect of angiotensin II receptor antagonist on insulin resistance, and the mechanism by which ACE inhibitor augments insulin sensitivity in both an animal model of insulin-resistant hypertension, fructose-fed rats (FFR), and human essential hypertensives (EHT).

METHODS

Study 1. Effects of ACE Inhibitor or Angiotensin II Antagonist in FFR *General Protocol* Six-week-old male Sprague-Dawley rats were used for all experiments. Prior to any manipulation, all rats were fed standard rat chow, containing 60% vegetable starch, 11% fat, and 29% protein. The rats were maintained on a 12-h light/dark (0600/1800) cycle. They were divided into two groups at the start of the study: those fed a standard chow (n = 11) or those given a fructose-rich chow (containing 66% fructose, 12% fat, and 22% protein; n = 30) for 4 weeks. FFR groups were then treated either with 1 mg/kg/day of TCV-116 orally (angiotensin II receptor antagonist; n = 13), 10 mg/kg of delapril orally (ACE inhibitor; n = 8), or vehicle (2.5% gum arabic solution; n = 9) for an additional 2 weeks.

Steady-State Plasma Glucose At the end of the drug administration period, rats were anesthetized with sodium pentobarbital and the right common carotid artery and the right jugular vein were exposed and cannulated with a PE50 for direct measurements of blood pressure, collecting blood samples, and administration of the infusate. The following day, in vivo insulin action was quantified by a modification of a method previously described.[13–16] Food was withdrawn at 0800 h the day of the study and the procedure was then started at 1400–1500 h. Rats received a continuous infusion of glucose (8 mg/kg/min) and insulin (2.5 mU/kg/min) simultaneously for 180 min. Implementation of this technique allowed for a comparable steady-state plasma glucose and insulin levels in all animals during the last hour of the study. Steady-state plasma glucose values were calculated from the mean of blood samples taken at 10-min intervals during the last 60 min of the infusion. Blood glucose levels were measured by the glucose oxidase method immediately after sampling.

Study 2. Effects of ACE Inhibitor or Angiotensin II Antagonist in EHT Thirteen essential hypertensives (EHT: WHO stage I–II, age 46.5 ± 4.1 years old, body mass index 24.4 ± 0.7 kg/m^2) and 18 age- and body-mass index-matched normotensive controls (NT: age 45.9 ± 4.1 years old, body mass index 23.1 ± 0.8 kg/m^2) were employed for the human study. They were inpatients and were kept on a constant diet containing 120 mEq of sodium and 75 mEq of potassium per day. After a 2-week control period, the 2-h euglycemic hyperinsulinemic glucose clamp according to DeFronzo et al[17] was carried out to estimate in vivo sensitivity for insulin in EHT and NT. In the glucose clamp study, blood was continuously withdrawn at 2.0 mL/h through a double-lumen catheter for glu-

cose analysis of arterialized blood. In addition, a contralateral antecubital vein was cannulated with a No. 18 plastic cannula for infusion of insulin and glucose.

Continuous insulin infusion, monitoring of glucose concentration, and infusion of variable amounts of glucose for clamping glucose levels at the basal state were performed with a model STG-22 artificial endocrine pancreas (Nikkiso Corp., Tokyo, Japan). The infusion rate of insulin (Actrapid Human, Novo Industries, Copenhagen, Denmark) was 40 mU/m^2 of body surface area/min. During insulin infusion, euglycemia was maintained by a variable infusion of a 20% glucose solution. The mean rate of glucose infusion for the last 30 min of the clamp was used as an indicator of insulin sensitivity (M-value): milligrams of glucose per square meter of body surface area per minute.[17] In the EHT group, 6 EHT (41.5 ± 5.9 years old, body mass index 24.9 ± 1.1 kg/m^2) were treated with delapril at 120 mg/day, and 7 (50.7 ± 5.6 years old, body mass index 24.0 ± 1.0 kg/m^2) were treated with 8 mg of TCV-116 daily for 2 weeks. Then, insulin sensitivity by the glucose clamp method was repeated in all patients. Plasma insulin level was measured as immunoreactive insulin level. The study was in accordance with the Declaration of Helsinki (1983). The study protocol was approved by the ethics committees of Sapporo Medical University. All patients gave written or verbal informed consent for all procedures.

Statistical Analysis All the data were expressed as mean ± SEM. Student's *t* test was used to determine significance in comparison of paired and unpaired data. To compare three or four groups, one-way analysis of variance was used. All calculated $P < .05$ were considered to indicate significance.

RESULTS

Study 1 The effect of the fructose-rich diet in Sprague-Dawley rats and the effect of delapril and TCV-116 in FFR on mean blood pressure are shown on Figure 1A. Blood pressure was significantly higher in FFR with vehicle (137.7 ± 3.8 mm Hg, $P < .05$) than in control (120.8 ± 2.7 mm Hg), and was lower in both delapril (108.1 ± 6.3 mm Hg, $P < .05$)- and TCV-116 (112.8 ± 4.1 mm Hg, $P < .05$)-treated groups than in FFR with vehicle. Figure 1B shows the insulin sensitivity assessed by the SSPG method in the FFR study. FFR with vehicle showed a significantly higher SSPG level (209.3 ± 7.6 mg/dL, $P < .05$) than the control group (136.8 ± 10.1 mg/dL). On the other hand, SSPG levels in both delapril (170.8 ± 4.2 mg/dL)- and TCV-116 (171.6 ± 6.8 mg/dL)-treated FFR were significantly lower ($P < .05$) than in FFR with vehicle, and not different from that in the control

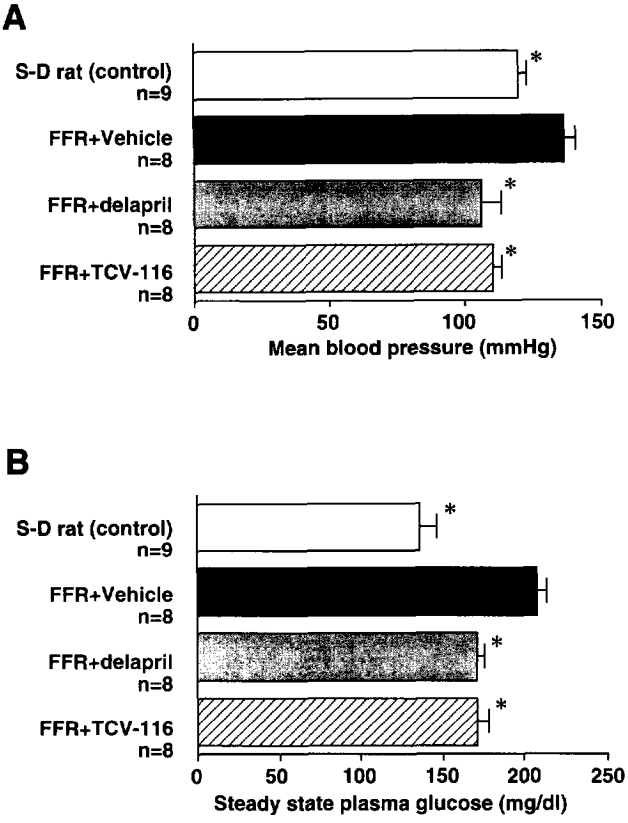

FIGURE 1. *Effect of delapril and TCV-116 on mean blood pressure (MBP)* **(A)** *and steady-state plasma glucose (SSPG)* **(B)** *in fructose-fed rats (FFR). *P < .05 compared with FFR.*

group. Furthermore, no difference was observed in SSPG between the delapril- and TCV-116–treated groups.

Study 2 Mean blood pressure was significantly higher in EHT (115.9 ± 4.8 mm Hg, $P < .001$) than in NT (90.0 ± 1.7 mm Hg). There was no difference in fasting blood sugar and insulin levels between EHT (89.2 ± 7.3 mg/dL and 4.3 ± 0.5 mU/L, respectively) and NT (96.2 ± 1.6 mg/dL and 2.4 ± 1.6 mU/L, respectively). However, the M-value in EHT (145.7 ± 15.3 mg/m^2/min) with the glucose clamp method was significantly lower ($P < .001$) than in NT (215.5 ± 50.7 mg/m^2/min) (Figure 2A). A 2-week treatment with delapril elicited a significant decrease in mean blood pressure (from 123.7 ± 7.8 to 111.5 ± 6.45 mm Hg, $P < .05$). TCV-116 also decreased blood pressure significantly in EHT (from 109.2 ± 4.8 to 93.2 ± 5.5 mm Hg, $P < .01$). There was no change in fasting blood sugar and plasma insulin level in either the delapril (from 82.8 ± 3.2 to 84.3 ± 3.8 mg/dL, and 4.3 ± 0.8 to 4.9 ± 1.2 mU/L, respectively)- or the TCV-116 (from 96.3 ± 13.0 to 94.4 ± 9.5 mg/dL, and from 4.3 ± 0.6 to 3.5 ± 0.3 mlU/L, respectively)-treated groups. Insulin sensitivity assessed by the M-value was signif-

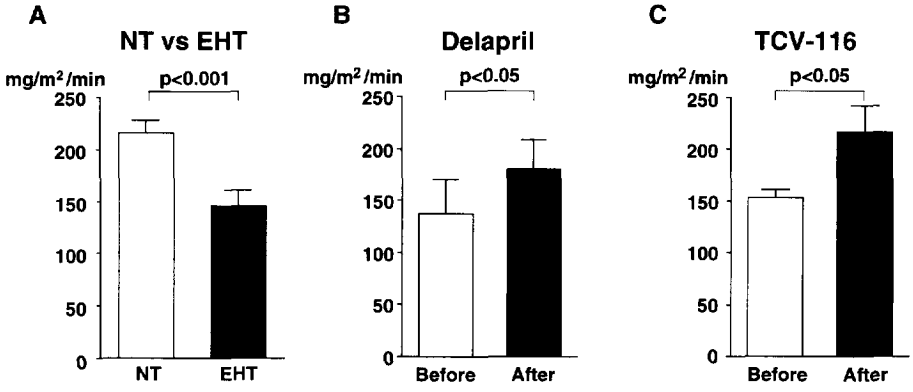

FIGURE 2. *M-values in normotensive subjects (NT) and patients with essential hypertension (EHT)* **(A)**, *and effects of delapril* **(B)** *and TCV-116* **(C)** *on insulin sensitivity (M-value) in essential hypertensives (EHT).*

icantly increased after treatment with delapril (from 137.3 ± 33.3 to 180.1 ± 28.2 mg/m^2/min, $P < .05$) (Figure 2B). Furthermore, TCV-116 also augmented the M-value significantly (from 152.9 ± 7.7 to 216.6 ± 25.5 mg/m^2/min, $P < .05$) to the same degree as in the delapril-treated group (Figure 2C).

DISCUSSION

Several recent observations have documented an association between hyperinsulinemia and hypertension in humans.[4–7,18–21] Several clinical studies have demonstrated that various ACE inhibitors improved insulin sensitivity in EHT.[5,7–10] In this study, we also demonstrated that the ACE inhibitor delapril improves insulin sensitivity in EHT and FFR. Regarding the mechanisms of improving insulin sensitivity by use of an ACE inhibitor, Rett et al[10] and Tomiyama et al[11] noted that an increased kinin level may contribute to this effect, possibly by reducing the breakdown of locally liberated kinin. However, the role of suppressed generation of angiotensin II by ACE inhibitor has not yet been clarified. It has been said previously that insulin resistance, hyperinsulinemia, and hypertriglyceridemia develop in a relatively short time when normal rats are fed a high-fructose diet.[13–16,22] In this study, the effect of the angiotensin II receptor antagonist on insulin sensitivity was examined using an insulin-resistant animal model, FFR.

We demonstrated that the angiotensin II receptor antagonist improves insulin resistance induced by fructose feeding in rats, as well as by ACE inhibition. These results indicate that angiotensin II antagonism may play an important role in the improvement of insulin sensitivity and the effects of the ACE inhibitor on insulin sensitivity may be caused to some extent by the suppression of angiotensin II generation. Although details of the mechanisms by which the angiotensin II receptor antagonist increases insulin sensitivity in FFR still remain unknown, increased muscular blood flow through the vasodilating action and suppression of norepinephrine release at the sympathetic nerve endings have been suspected. Regarding the effects of the angiotensin II receptor antagonist on insulin sensitivity, Tomiyama et al[11] reported that the angiotensin II receptor antagonist losartan lowered blood pressure but did not affect the glucose infusion rate in spontaneously hypertensive rats. The discrepancy between their results and ours suggests that the improving effects of angiotensin II receptor antagonist on insulin sensitivity might be greater in the insulin-resistant animal model.

This is the first report to demonstrate that the angiotensin II receptor antagonist improves insulin resistance in EHT. In our study, the same extent of improvement of insulin sensitivity was observed in both the ACE inhibitor and angiotensin II antagonist in EHT. These results indicated that the suppression of the angiotensin II action might be concerned with the improvement of insulin sensitivity even in EHT, as well as in FFR.

The renin-angiotensin system is involved in the pathogenesis of cardiovascular disease by regulating blood pressure through the production of angiotensin II, a potent vasoconstrictor which also stimulates smooth-muscle cell proliferation.[23,24] Recently, it has been suggested that insulin resistance which leads to hyperinsulinemia may be associated with hypertensive arteriosclerotic complications, including coronary heart disease,[1–4] and that metabolic side effects should be considered while selecting the antihypertensive agents.[25,26] TCV-116, a newly synthesized angiotensin II receptor antagonist with no agonistic properties, attenuated the magnitude of the blood pressure elevation and improved insulin resistance as assessed by SSPG in FFR and as assessed by the glucose clamp method in EHT. These results suggest that angiotensin II antagonist and ACE inhibitors should be very useful antihypertensive drugs in EHT with insulin resistance.

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