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Effects of losartan in combination with or without exercise on insulin resistance in Otsuka Long–Evans Tokushima Fatty rats

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Abstract

Hypertension often complicates type 2 diabetes mellitus, and angiotensin converting enzyme inhibitor treatment has been shown to improve insulin resistance in such cases. However, the effect of angiotensin II type-1 (AT_1) receptor antagonists on insulin resistance is still controversial. To gain further information on this effect, we examined the effect of losartan on insulin resistance in Otsuka Long–Evans Tokushima Fatty (OLETF) rats, a model of type 2 diabetes mellitus. Losartan administration alone lowered systolic blood pressure, but did not improve oral glucose tolerance test or insulin resistance in OLETF rats. However, the administration of losartan with exercise significantly improved both systolic blood pressure and insulin resistance relative to control OLETF rats. On the other hand, losartan treatment, regardless of exercise, increased glucose uptake in excised soleus muscle and fat cells. To explore the beneficial effect of losartan on skeletal muscle glucose uptake, we examined intracellular signaling of soleus muscle. Although Akt activity and glucose transporter type 4 (GLUT4) expressions were not affected by losartan with or without exercise, extracellular signal-regulated kinase (ERK1/2) and p38 mitogen-activated protein (MAP) kinase activities were increased by both interventions. These results indicate that angiotensin AT_1 receptor antagonist improved local insulin resistance, but not systemic insulin resistance. These findings may explain the controversy over the effect of angiotensin AT_1 receptor antagonists on insulin resistance in clinical use. The enhancing effect of angiotensin AT_1 receptor antagonist on skeletal muscle glucose uptake may be attributable to MAP kinase activation or other mechanisms rather than phosphatidylinositol 3-kinase activation.

Keywords: Insulin resistance; Angiotensin; MAP (mitogen-activated protein) kinase; OLETF (Otsuka Long–Evans Tokushima Fatty), rat

1. Introduction

Diabetes mellitus is a disorder characterized by an elevated blood glucose level due to insufficient insulin action, and is classified into two groups, type 1 and type 2. Type 2 diabetes is commonly attributable to insulin resistance, which can be caused by numerous factors including unknown genetic factors, obesity, a high fat-diet, and insufficient physical exercise (Daly et al., 1997; Elson and Meredith, 1998; Goodyear and Kahn, 1998; Kaplan, 1998). Patients with type 2 diabetes often have hypertension (National High Blood Pressure Education Program Working Group, 1994; Sowers and Epstein, 1995), and insulin resistance has been reported to be a risk factor for cardiovascular diseases (Manson et al., 1991; Reaven, 1991). Ramipril, an angiotensin converting enzyme inhibitor, was beneficial for cardiovascular events and overt nephropathy in patients with diabetes (Heart Outcomes Prevention Evaluation (HOPE) Study Investigators, 2000). Another angiotensin converting enzyme inhibitor, imidapril, improved the impaired insulin sensitivity in Zucker fatty rats (Nawano et al., 1999). However, it is controversial whether the angiotensin II type-1 (AT_1) receptor antagonist improves

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insulin sensitivity in type 2 diabetes mellitus (Fogari et al., 1998; Moan et al., 1995, 1996; Tomiyama et al., 1994).

Otsuka Long–Evans Tokushima Fatty (OLETF) rats are a newly developed model of human type 2 diabetes (Kawano et al., 1992). OLETF rats develop not only glomerulosclerosis but also cardiac complications, making them a useful model to study the pathogenesis of cardiac and renal complications in type 2 diabetes mellitus (Yagi et al., 1997). It has been reported that insulin-induced intracellular signaling was decreased in OLETF rats (Ishizuka et al., 1998). Insulin-induced intracellular signaling events have been investigated extensively in recent years. Upon the binding of insulin to its receptor, the intrinsic tyrosine kinase of the insulin receptor β subunit is activated, and insulin signaling pathways diverge. One pathway proceeds through the insulin receptor substrates IRS-1 and IRS-2 and depends on activation of the enzyme phosphatidylinositol 3-kinase. The other pathway proceeds through Grb2/son of sevenless (Sos) and Ras, leading to activation of the mitogen-activated protein (MAP) kinase isoforms extracellular signal-regulated kinase (ERK1/2) (Cusi et al., 2000).

In the present study, we investigated the chronic effect of losartan, an angiotensin AT_1 receptor antagonist, on insulin resistance in OLETF rats. We also examined whether exercise alters the effect of losartan on insulin resistance. Moreover, we investigated whether losartan treatment affects glucose uptake and intracellular signaling in fat cells and soleus muscle of OLETF rats.

2. Materials and methods

2.1. Chemicals

Losartan was purchased from E.I. du Pont de Nemours and Company (Wilmington, DE). Phospho-ERK 1/2 antibody (Thr202/Tyr204), Phospho-p38 MAP kinase antibody (Thr180/Tyr182) and Phospho-Akt (Ser473) antibody were purchased from New England Biolabs (Bevery, MA). Anti-glucose transporter type 4 (GLUT4) antibody was prepared by immunizing rabits with keyhole limpet hemocyanin-coupled peptide of COOH-terminal 30 amino acids of GLUT4 as described previously (Imanaka et al., 1998). All other chemicals were of reagent grade, from commercial sources and used without further purification.

2.2. Animals and experimental design

All animal care and treatments were conducted in accordance with the guidelines of the animal use and care committee of the University of Tokushima. All the experiments were performed according to the Guide for the Care and Use of Laboratory Animals published by US National Institutes of Health (NIH publication No. 85-23, revised 1985). The animals used in this study were 20 male non-insulin dependent diabetic (OLETF) rats provided by the Tokushima Research Institute (Otsuka Pharmaceutical Tokushima, Japan). Four-week-old OLETF rats were housed singly at constant room temperature (22 ± 2 °C) with a 12-h light/dark cycle. Rats were fed standard rat chow including 5% fat (Oriental Yeast, Tokyo, Japan). Food and water were available ad libitum and rats grew satisfactorily. At the age of 26 weeks, they were randomly assigned to four groups: to be given a normal diet (OLETF-control group), a losartan-added diet (losartan treated group), a normal diet with exercise (exercise alone group) or the losartan-added diet with exercise (losartan + exercise group). Animals of the exercise alone group and the losartan + exercise group were placed in individual cages with an exercise wheel (Nishin, Tokushima, Japan) and allowed to run at their own pace (150–1000 m/day). Body weight and fasting blood glucose concentrations were not significantly different among groups at the beginning of the experiment. Losartan (1 mg/kg/day) was added to the diet from 26 to 30 weeks of age.

2.3. Oral glucose tolerance test and euglycemic hyperinsulinemic glucose clamp

At 30 weeks of age, rats underwent an oral glucose tolerance test after an overnight fast. Two grams of glucose per kilogram of body weight was administered orally. Blood was drawn from a tail vein at 0, 30, 60, and 120 min for measurement of plasma glucose concentrations. Insulin-mediated whole-body glucose uptake was determined in 30-week-old anesthetized rats by using an euglycemic insulin clamp (Kergoat and Portha, 1985). After overnight food deprivation, rats were anesthetized by intraperitoneal injection of pentobarbital (50 mg/kg), and catheters were inserted into the carotid artery and jugular vein. Rats received a 1-h infusion of insulin (60 pmol/kg min). A glucose solution (100 g/l) was initiated at time 0; the rate was adjusted to maintain the plasma concentration of glucose at 6.1 mmol/l. Total body glucose uptake represents the mean glucose infusion rate during the last 20 min. Before the beginning of the clamp study, blood samples were collected from the carotid artery. The plasma levels of glucose were measured by the glucose oxidase method (Toecho Super, Kyoto Daiichi Kagaku, Kyoto, Japan). The plasma level of insulin was measured by a standard radioimmunoassay technique using a commercial kit (Eiken kagaku, Tokyo, Japan) with rat insulin used as the standard (Novo, Bagsvared, Denmark).

2.4. Glucose uptake assay

At 30 weeks of age, the rats were sacrificed, and soleus muscle and the intra-abdominal fat pads (mesenteric, epididymal, and retroperitoneal fat) were surgically removed and weighed. Measurements of 2-[^{3}H]deoxyglucose uptake in fat cells were previously described by Hayashi et al.

(1998). The soleus muscle preparation was rinsed with Krebs–Ringer bicarbonate–HEPES buffer (KRBH; in mM: 140 NaCl, 2.5 $MgSO_4$, 1 $CaCl_2$, and 5 KCl, KH_2PO_4, 24.6 $NaHCO_3$, 30 HEPES, pH 7.4) in 5% bovine serum albumin. The muscles were rinsed again in 1 ml of 5% bovine serum albumin/Krebs–Ringer bicarbonate buffer (KRBB; in mM: 140 NaCl, 2.5 $MgSO_4$, 1 $CaCl_2$, and 5 KCl, KH_2PO_4, 24.6 $NaHCO_3$, pH 7.4) containing 8 mM D-mannitol at 37 °C for 10 min, and then glucose uptake was quantitated by exposing the skeletal muscle to 1 mM 2-[^{3}H] deoxyglucose (1.5 μCi/ml) and 0.1 M non-radio labeled 2-deoxyglucose at 37 °C for 3 min. Nonspecific uptake was determined by quantitating cell-associated radioactivity in the presence of 1 M cytochalasin B on ice. The uptake buffer was removed rapidly and the skeletal muscle were lysed in 300 μl of 1 N NaOH at 80 °C for 10 min. Digestates were neutralized with 300 μl of 1 N HCl, and particulates were precipitated by centrifuging at $16{,}000 \times g$ for 10 min. The associated radioactivity in the supernatant was determined by liquid scintillation counting.

2.5. Preparation of cell lysates for MAP kinase activity assay

Soleus muscle was homogenized in lysis buffer containing 20 mM Tris · HCl, pH 8.0, 140 mM NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 1% NP-40, 1 mM dithiothreitol, 10% glycerol, 0.5 mM sodium orthovanadate, and 20 μM phenylmethylsulfonyl fluoride. The lysates were then sonicated (Handy Sonic UR-20 P, Tomy Seiko Tokyo, Japan) on ice for 1 min and were transferred to microcentrifuge tubes and centrifuged at $16{,}000 \times g$ for 20 min at 4 °C. The protein concentrations of the supernatants were measured with a protein assay kit (Bio-Rad Protein Assay, Bio-Rad, USA) and the samples stored at −80 °C until MAP kinase assay.

2.6. Measurements of ERK 1/2 and p38 MAP kinase activities in soleus muscle

For immunoblot analysis, cell lysates were subjected to sodium dodecylsulfate (SDS)-polyacrylamide gel electrophoresis, and proteins were transferred to nitrocellulose membranes (Hybond™-ECL, Amersham Pharmacia Biotech, Buckinghamshire, England) as described previously (Yoshizumi et al., 2000). The membrane was blocked for 1 h at room temperature with a commercial blocking buffer from Amersham Pharmacia Biotech. The blots were then incubated for 12 h with anti-phosphospecific ERK1/2 or p38 MAP kinase antibodies (New England Biolabs), followed by incubation for 1 h with secondary antibody (horseradish peroxidase conjugated). Immunoreactive bands were visualized using enhanced chemiluminescence (ECL, Amersham Pharmacia Biotech) and were quantified by densitometry in the linear range of film exposure using a UMAX Astra 2200 scanner and NIH image 1.60.

2.7. JNK activity assay

c-Jun amino terminal kinase (JNK) activity was measured with a commercially available kit based on phosphorylation of recombinant c-Jun (New England Biolabs). After treatment, cells were rinsed twice with ice-cold phosphate-buffered saline (PBS), scraped off the plates into lysis buffer (included in the kit), and sonicated three times on ice. After the cell debris had been removed by centrifugation ($16{,}000 \times g$, 20 min, 4 °C), the protein content in the supernatant was measured using a protein assay kit (PIERCE). Equal amounts of protein (300 μg) were then immunoprecipitated with c-Jun (1–89) fusion protein beads overnight. Next the beads were washed, and kinase assays were performed according to the instructions of the manufacturer. The beads were then loaded on a 10% SDS-polyacrylamide gel, and immunoblotting was performed with an antibody against phosphospecific c-Jun (Yoshizumi et al., 2000).

2.8. AKt activity and Western blot analysis of GLUT4 protein expression in the soleus muscle

The cell lysates from soleus muscle were subjected to 10% SDS-polyacrylamide gel electrophoresis and immunoblotted with an anti-Akt antibody or phosphospecific Akt (Ser473) antibody (New England Biolabs). GLUT4 protein expression was determined by immunoblotting with anti-GLUT4 antibody as described in Section 2.1.

2.9. Statistical analysis

Values are reported as the mean ± S.D. from experiments done in triplicate. Statistical analysis was performed with the Stat View 5.0 package (ABACUS Concepts, Berkeley, CA). Differences were analyzed with an unpaired two-tailed Student's *t*-test or Welch's *t*-test as appropriate and values at $P < 0.05$ were accepted as significant.

3. Results

3.1. Body weight, heart rate, blood pressure, and plasma levels of glucose and insulin

Effects of losartan with or without exercise on body weight, heart rate, blood pressure, and plasma levels of glucose and insulin in OLETF rats are shown in Table 1. There is no significant difference in body weight between those with and without losartan administration from 26 to 30 weeks of age. Exercise tended to decrease body weight,

Table 1
Effects of losartan with or without exercise on body weight, heart rate, blood pressure, plasma levels of glucose and insulin in OLETF rats

	Control	Losartan	Losartan + exercise
Body weight (g)			
26 weeks of age	662.8 ± 40.3	649.6 ± 33.0	637.5 ± 73.7
30 weeks of age	644.9 ± 35.6	651.2 ± 34.4	527.5 ± 70.7
Heart rate (beats/min)	391 ± 8	403 ± 13	367 ± 24
Systolic blood pressure (mm Hg)	163.6 ± 5.3	151.3 ± 5.2[a]	144.3 ± 8.0[a]
Diastolic blood pressure (mm Hg)	111.8 ± 8.3	100.5 ± 2.9[a]	100.0 ± 4.5[a]
Glucose (mg/dl)	118 ± 17	118 ± 11	108 ± 26
Insulin (ng/ml)	7.06 ± 2.30	6.25 ± 1.15	2.91 ± 1.24[a]

Data are means ± S.D.
[a] $P < 0.05$ vs. control.

although it did not reach the level of statistical significance in OLETF rats. Heart rates were similar among the groups. However, systolic and diastolic blood pressures were significantly lower for both losartan with or without exercise than the control. Concentrations of plasma glucose did not differ among the groups. Plasma insulin concentrations were significantly lower in the group with exercise than in the other groups.

3.2. Glucose tolerance and in vivo glucose disposal

The results of glucose tolerance did not differ significantly between the control and losartan treatment groups. Blood glucose concentrations after glucose administration, however, were significantly higher in the control group and the losartan-treated group than in the groups of exercise alone and losartan with exercise (Fig. 1). There was no significant difference in glucose infusion rate between the control and the losartan-administered groups. However, glucose infusion rate was significantly higher in the exercise alone group and the losartan with exercise group than the other two groups. Losartan supplementation to exercise showed a tendency of an additive effect to exercise alone in the glucose infusion rate; however, there was no significant difference between the effects of exercise alone and losartan with exercise on the glucose infusion rate.

3.3. Effects of losartan administration with or without exercise on 2-deoxyglucose uptake in soleus muscle and fat cells from OLETF rats

Fig. 2A shows the effects of losartan administration with or without exercise on the uptake of 2-[^{3}H] deoxyglucose into soleus muscle. Both losartan administration with or without exercise caused an increase in glucose uptake in skeletal muscle, although the increase was greater with exercise, i.e. 1.21-fold and 1.57-fold the control 2-deoxyglucose uptake level, respectively. 2-[^{3}H] deoxyglucose uptake in fat cells showed similar results to those in soleus muscle (Fig. 2B). It was observed that both losartan treatment with or without exercise stimulated increases in glucose uptake in fat cells (1.81-fold and 2.49-fold the control 2-deoxyglucose uptake level, respectively).

3.4. Effects of losartan administration with or without exercise on Akt phosphorylation and GLUT4 protein expression in the soleus muscle from OLETF rats

It was shown that activation of Akt, a serine–threonine kinase, is involved in insulin-stimulated glucose uptake (Duronio et al., 1998; Hajduch et al., 1998). Therefore, we next investigated the effects of losartan and exercise on Akt phosphorylation in soleus muscle. As shown in Fig. 3, regardless of exercise, losartan had no effect on Akt

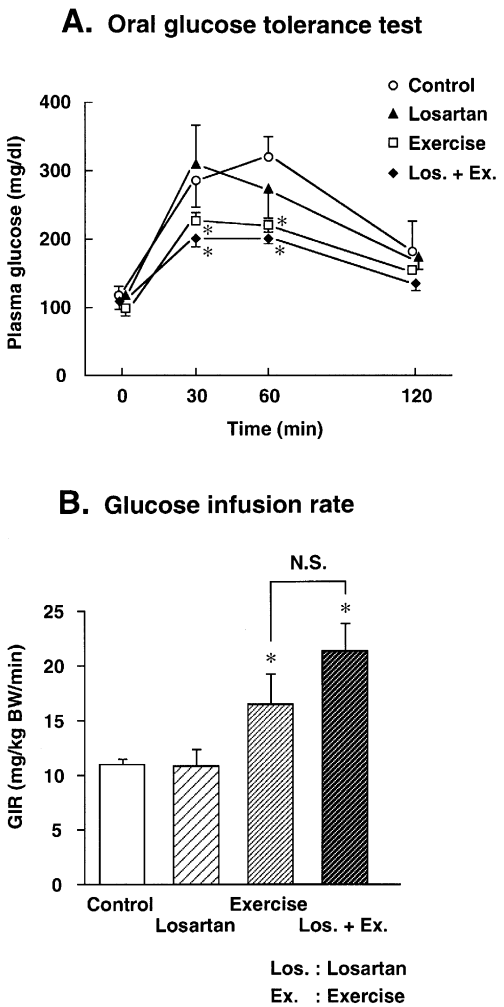

Fig. 1. Mean (± S.D.) blood glucose concentrations before and after an oral glucose tolerance test (A) and glucose infusion rates (B) in OLETF rats (control group, losartan administration group, exercise alone group and losartan with exercise group). The asterisks represent significant differences compared with the control group ($^*P < 0.05$). N.S., not significant.

phosphorylation. Activation of phosphatidylinositol 3-kinase, which is upstream of Akt, was not observed either (data not shown). These results demonstrate that losartan administration with or without exercise had no effect on the phosphatidylinositol 3-kinase pathway. In addition, we also examined the expression of GLUT4 protein in the soleus muscle because GLUT4 activation was reported to be important to glucose transport into cells (Kishi et al., 1998). However, no significant difference was observed in the expression of GLUT4 by losartan treatment with or without exercise compared with the controls.

3.5. Effects of losartan administration with or without exercise on MAP kinase activation in soleus muscle from OLETF rats

Losartan administration alone significantly increased ERK1/2 and p38 MAP kinase activation in soleus muscle compared to the control (3.40-fold and 1.80-fold, respectively) (Fig. 4A,B). Losartan administration with exercise also activated ERK1/2 and p38 MAP kinase in soleus muscle (3.83-fold and 1.77-fold, respectively) (Fig. 4A,B).

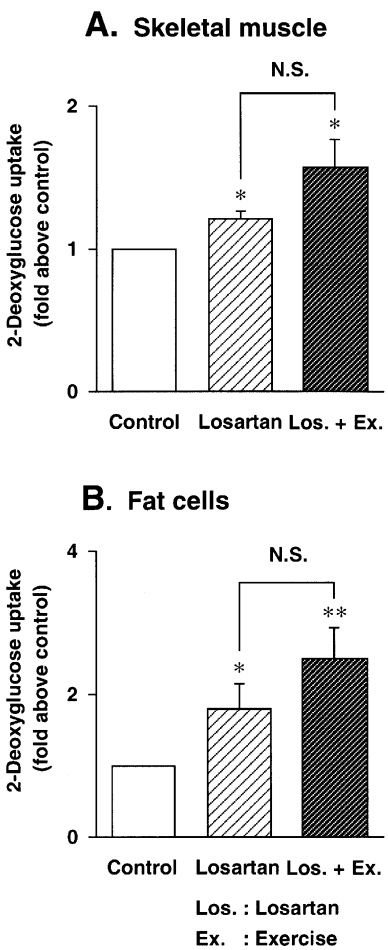

Fig. 2. Effects of losartan administration with or without exercise on 2-deoxyglucose uptake in soleus muscle (A) and fat cells (B). Values are the means ± S.D. of at least three individual experiments. The asterisks represent significant differences compared with the control group ($^{*}P < 0.05$, $^{**}P < 0.01$). N.S., not significant.

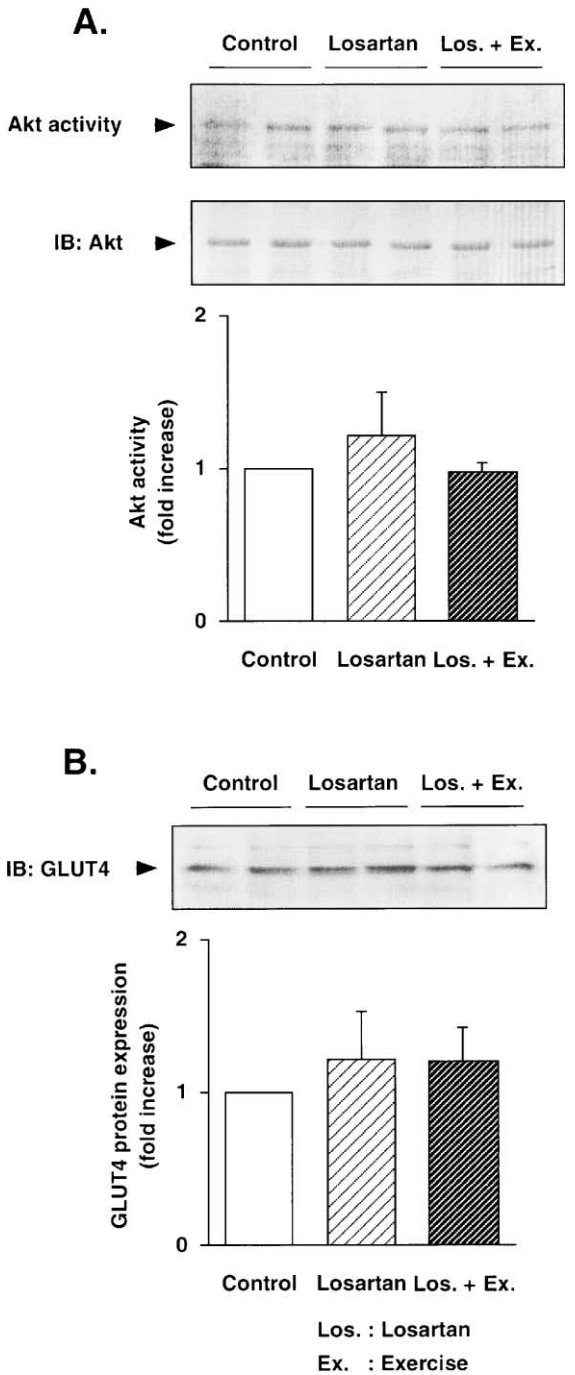

Fig. 3. Effects of losartan administration with or without exercise on Akt phosphorylation (A) and GLUT4 protein expression (B) in soleus muscle from OLETF rats. The activity of Akt and GLUT4 protein expression was measured as described in Section 2. No difference in the amount of Akt was observed in samples by Western blot analysis with Akt antibodies (middle panel in A). GLUT4 protein expression was determined by Western blot analysis with anti-GLUT4 antibodies. Values were normalized by arbitrarily setting the densitometry of control to 1.0 (values are the mean ± S.D., $n = 4$).

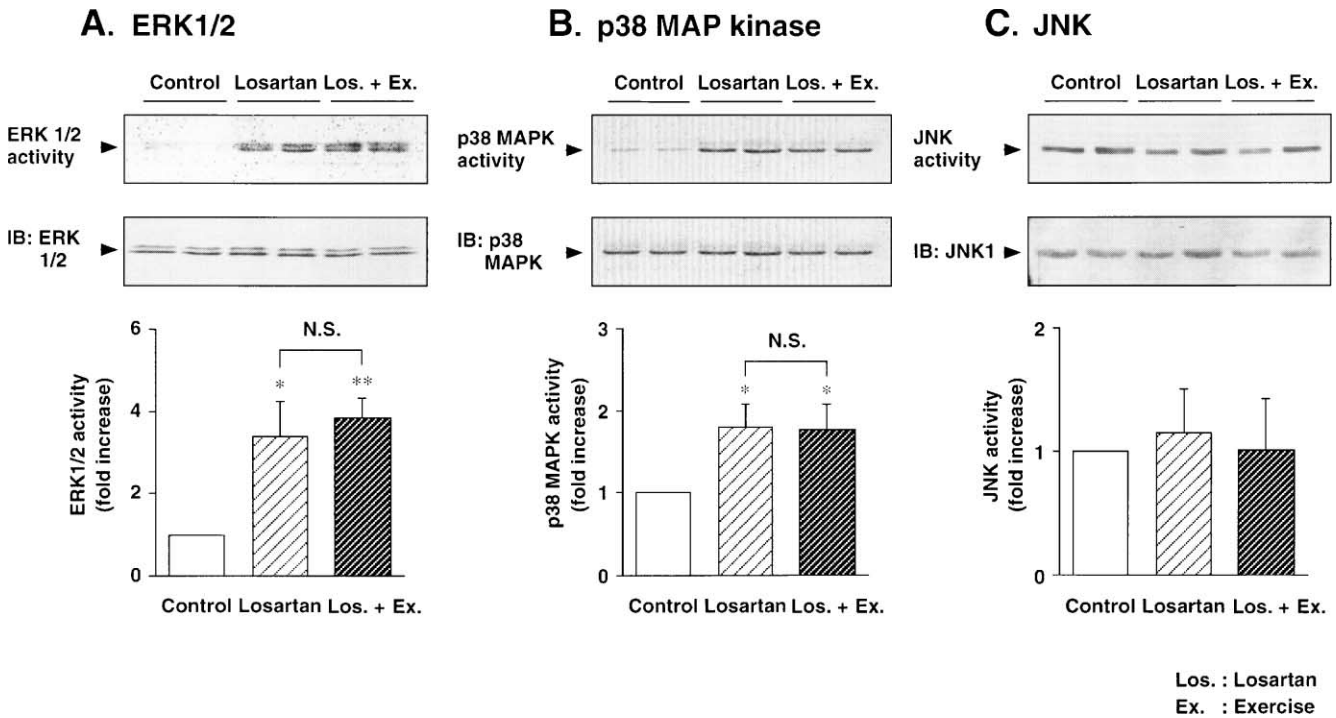

Fig. 4. Effects of losartan administration with or without exercise on MAP kinases (ERK1/2 (A), p38 MAP kinase (B) and JNK (C)) activities in soleus muscle from OLETF rats. No difference in the amounts of ERK1/2, p38 MAP kinase and JNK was observed in samples by Western blot analysis with ERK1/2, p38 MAP kinase and JNK antibodies (middle panels). Values were normalized by arbitrarily setting the densitometry of control to 1.0 (values are the mean ± S.D., $n = 4$). The asterisks represent significant differences compared with the control group ($^{*}P < 0.05$, $^{**}P < 0.01$). N.S., not significant.

In contrast, losartan administration with or without exercise had no effect on JNK activity (Fig. 4C).

4. Discussion

The major findings of this study are that losartan administration lowered systolic blood pressure, but not oral glucose tolerance test or glucose infusion rate, an index of insulin resistance, in OLETF rats. In contrast, losartan with exercise significantly improved not only systolic blood pressure but also oral glucose tolerance test and glucose infusion rate in the rats. Regardless of its combination with exercise, losartan increased glucose uptake in both skeletal muscle and fat cells. The intracellular signaling mechanism of the increase in glucose uptake was examined, and it was found that the phosphatidylinositol 3/Akt-kinase pathway, which is known as a major signaling pathway for glucose uptake, was not affected by losartan administration with or without exercise in soleus muscle extracts. In contrast, both losartan treatment with and without exercise activated MAP kinase family members, ERK1/2 and p38 MAP kinase, in OLETF rats.

Previous studies showed that angiotensin converting enzyme inhibitor contributed to improve insulin sensitivity in rats (Carvalho et al., 1997; Nawano et al., 1999; Tomiyama et al., 1994). Moreover, treatment with cilnidipine, which is a long acting dihydropiridine Ca^{2+} channel antagonist, had a beneficial effect on insulin resistance together with the antihypertensive effect in OLETF rats (Harada et al., 1999). However, it is controversial whether insulin sensitivity in type 2 diabetes mellitus is improved by angiotensin AT_1 receptor antagonists, which lower blood pressure through a different mechanism from angiotensin converting enzyme inhibitors and Ca^{2+} channel antagonists (Moan et al., 1995, 1996; Tomiyama et al., 1994). In the present study, losartan administration improved systolic blood pressure in OLETF rats, but had no effect on the systemic insulin sensitivity of the peripheral tissues as evaluated by glucose infusion rate. Since the dose of losartan used in the present study may be low compared with other cardiovascular investigations, the possibility cannot be denied that the lack of an improving effect of losartan on the oral glucose tolerance test and the glucose infusion rate may be attributable to its low dose treatment. However, it was reported that 1 week administration of low dose losartan (approximately 2 mg/day) improved high blood pressure, whereas it did not affect insulin sensitivity in spontaneously hypertensive rats (Tomiyama et al., 1994). In addition, it was reported that 50-mg administration of losartan to hypertensive patients for 4 weeks lowered blood pressure but did not affect insulin sensitity or glucose metabolism (Moan et al., 1996). Since losartan lowered blood pressure in OLETF rats in

the present study even though the used dose was low, it was suggested that losartan was effective at least for high blood pressure in OLETF rats. Further studies are needed to define the high dose effect of losartan on insulin resistance in OLETF rats.

Since angiotensin converting enzyme inhibitor, angiotensin AT_1 receptor antagonist and Ca^{2+} channel antagonist all decrease blood pressure through vasodilation, it is suggested that the beneficial effects of the angiotensin converting enzyme inhibitor and Ca^{2+} channel antagonist on insulin resistance were probably due to other factors rather than a simple increase in blood flow. For an explanation of the beneficial effect of the Ca^{2+} channel antagonist on insulin resistance, it was reported that cilnidipine was effective in improving insulin resistance through the blocking action on N-type Ca^{2+} channels, which in turn suppressed the release of norepinephrine from the end of sympathetic neurons and the reflexive increase in sympathetic tone (Harada et al., 1999). It was also reported that long-term activation of sympathetic function worsens insulin resistance (Julius and Jamerson, 1994). One possible explanation for the discrepancy of the effect between angiotensin converting enzyme inhibitor and angiotensin AT_1 receptor antagonist on insulin resistance is that the increase in bradykinin production by angiotensin converting enzyme inhibitor treatment inhibits dephosphorylation of the insulin receptor, which results in an enhancement of insulin receptor phosphorylation and subsequent activation of phosphatidylinositol 3-kinase, one of the key kinases for glucose metabolism (Carvalho et al., 1997; Henriksen and Jacob, 1995). However, since we did not measure bradykinin in plasma directly in this study, we cannot exclude other possibilities. In addition, the discrepancy between the effects of angiotensin converting enzyme inhibitors and losartan on insulin resistance should also be considered from the aspect of the dosage of the drug used.

Although the systemic effect of losartan on insulin resistance is still controversial, the local effect of losartan in each organ, which utilizes glucose mainly for metabolism, is not well characterized. In the present study, we found that losartan caused an increase in glucose uptake in skeletal muscle and fat cells. It is difficult to explain the discrepancy of the systemic and local effect of losartan on insulin sensitivities. However, one possibility is that an unidentified inhibitory substance in blood affects systemic insulin sensitivity in OLETF rats, because of the absence of influence of blood contents in experiments with isolated skeletal muscle and fat cells. Another possible explanation is that treatment with angiotensin AT_1 receptor antagonist causes an increase in the plasma Ang II level, which probably stimulates angiotensin AT_2 receptor (Bernstein and Alexander, 1992). Stimulation of angiotensin AT_2 receptor may exert G_i protein activation, and result in an increase in glucose uptake via GLUT4 translocation (Wang et al., 2000). Our findings firstly showed that the effect of losartan on insulin resistance differs between systemic and local in OLETF rats. Although the underlying mechanism of the difference is unexplained at present, the present findings suggest that the discrepancy between the local and systemic effects of losartan may explain previous contradictory findings. Losartan administration with exercise, however, improved significantly both systemic and local insulin sensitivities. Of course, the experiments with samples from exercise-alone OLETF rats would be informative to define the effect of exercise itself on glucose uptake; however, due to the lack of samples we could not perform it. There is overwhelming evidence that physical exercise can reduce the risk of developing cardiovascular diseases and several other conditions that are known to be significant complications associated with type 2 diabetes mellitus (Goodyear and Kahn, 1998; Uusitupa, 1996). Thus, continuous physical exercise would be an important adjunct to the treatment of type 2 diabetes mellitus.

The intracellular signaling mechanisms after insulin receptor stimulation have been investigated extensively in recent years. The Akt/phosphatidylinositol 3-kinase pathway has been recognized as a major pathway in insulin signaling. In the present study, we found that Akt was not activated in skeletal muscle by losartan treatment with or without exercise, although glucose uptake was increased in each setting. These results are surprising because activation of Akt is believed to cause an increase in glucose uptake (Duronio et al., 1998; Hajduch et al., 1998). However, since it has been reported that exercise and muscle contraction did not stimulate phosphatidylinositol 3-kinase signaling (Goodyear et al., 1995), exercise itself may not affect Akt activity in skeletal muscle. The influence of losartan on phosphatidylinositol 3-kinase remains to be elucidated. It was reported that GLUT4 activation was important for glucose transport into cells (Kishi et al., 1998). We, therefore, examined the expression of GLUT4 protein in the soleus muscle and found that there were no significant differences in the expression of GLUT4 among the control, losartan and losartan with exercise groups. Since we previously reported that GLUT4 membrane translocation was important for its activation, losartan treatment may facilitate GLUT4 translocation rather than induce its expression within the cells, although we have no direct evidence of this.

It was reported that exercise stimulated ERK1/2 and p38 MAP kinase intracellular signaling cascades in rat skeletal muscle (Goodyear et al., 1996). We also found that activities of ERK1/2 and p38 MAP kinase were increased by losartan alone as well as in combination with exercise, whereas JNK activation was not observed. Based on the above findings, we considered that glucose uptake in skeletal muscle and fat cells might be stimulated by MAP kinase including ERK1/2 and p38 MAP kinase or other mechanisms, but not by phosphatidylinositol 3-kinase. Some previous reports support our findings. It has been reported that microinjection of MAP kinase increased the

transport of glucose into *Xenopus* oocytes (Merrall et al., 1993). Sweeney et al. (1999) reported that an inhibitor of p38 MAP kinase, 4-(4-fluorophenyl)-2-(4-methyl-sulfinyl-phenyl)-5-(4-pyridyl)1*H*-imidazole (SB203580), could prevent insulin-stimulated glucose uptake in 3T3-L1 adipocytes and L6 muscle cells. They described that the inhibitory effect of SB203580 was attributed to its inhibitory effect on glucose transport via the inhibition of insulin-dependent p38 MAP kinase activation. Since the existence of p38 MAP kinase-mediated, but phosphatidylinositol 3-kinase-independent, hexose transport has been demonstrated (Tong et al., 2000), it is reasonable to conclude that the increase in glucose uptake in skeletal muscle may be due to the activation of ERK1/2 and p38 MAP kinase not phosphatidylinositol 3-kinase.

In conclusion, losartan administration improved systolic blood pressure, but not insulin resistance in OLETF rats. However, losartan treatment with exercise significantly decreased systolic blood pressure and improved insulin resistance. In contrast, losartan treatment with or without exercise increased glucose uptake in both skeletal muscle and fat cells from OLETF rats. Although the discrepancy between systemic (oral glucose tolerance test and glucose infusion rate) and local (glucose uptake in exercised muscle and fat preparations) effects of losartan is unexplained at present, it was suggested that the increase in local glucose uptake is attributable to the activation of ERK1/2 and p38 MAP kinase, or some other mechanism, but not phosphatidylinositol 3-kinase. Our findings may provide information to reveal the true effect of losartan on insulin resistance in type 2 diabetes mellitus.

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References

Bernstein, K.E., Alexander, R.W., 1992. Counterpoint: molecular analysis of the angiotensin II receptor. Endocr. Rev. 13, 381–386.

Carvalho, C.R., Thirone, A.C., Gontijo, J.A., Velloso, L.A., Saad, M.J., 1997. Effect of captopril, losartan, and bradykinin on early steps of insulin action. Diabetes 46, 1950–1957.

Cusi, K., Maezono, K., Osman, A., Pendergrass, M., Patti, M.E., Pratipanawatr, T., DeFronzo, R.A., Kahn, C.R., Mandarino, L.J., 2000. Insulin resistance differentially affects the PI 3-kinase- and MAP kinase-mediated signaling in human muscle. J. Clin. Invest. 105, 311–320.

Daly, M.E., Vale, C., Walker, M., Alberti, K.G., Mathers, J.C., 1997. Dietary carbohydrates and insulin sensitivity: a review of the evidence and clinical implications. Am. J. Clin. Nutr. 66, 1072–1085.

Duronio, V., Scheid, M.P., Ettinger, S., 1998. Downstream signalling events regulated by phosphatidylinositol 3-kinase activity. Cell Signalling 10, 233–239.

Elson, D.F., Meredith, M., 1998. Therapy for type 2 diabetes mellitus. Wmj 97, 49–54.

Fogari, R., Zoppi, A., Lazzari, P., Preti, P., Mugellini, A., Corradi, L., Lusardi, P., 1998. ACE inhibition but not angiotensin II antagonism reduces plasma fibrinogen and insulin resistance in overweight hypertensive patients. J. Cardiovasc. Pharmacol. 32, 616–620.

Goodyear, L.J., Kahn, B.B., 1998. Exercise, glucose transport, and insulin sensitivity. Annu. Rev. Med. 49, 235–261.

Goodyear, L.J., Giorgino, F., Balon, T.W., Condorelli, G., Smith, R.J., 1995. Effects of contractile activity on tyrosine phosphoproteins and PI 3-kinase activity in rat skeletal muscle. Am. J. Physiol. 268, E987–E995.

Goodyear, L.J., Chang, P.Y., Sherwood, D.J., Dufresne, S.D., Moller, D.E., 1996. Effects of exercise and insulin on mitogen-activated protein kinase signaling pathways in rat skeletal muscle. Am. J. Physiol. 271, E403–E408.

Hajduch, E., Alessi, D.R., Hemmings, B.A., Hundal, H.S., 1998. Constitutive activation of protein kinase B alpha by membrane targeting promotes glucose and system A amino acid transport, protein synthesis, and inactivation of glycogen synthase kinase 3 in L6 muscle cells. Diabetes 47, 1006–1013.

Harada, N., Ohnaka, M., Sakamoto, S., Niwa, Y., Nakaya, Y., 1999. Cilnidipine improves insulin sensitivity in the Otsuka Long–Evans Tokushima fatty rat, a model of spontaneous NIDDM. Cardiovasc. Drugs Ther. 13, 519–523.

Hayashi, T., Hirshman, M.F., Kurth, E.J., Winder, W.W., Goodyear, L.J., 1998. Evidence for 5′ AMP-activated protein kinase mediation of the effect of muscle contraction on glucose transport. Diabetes 47, 1369–1373.

Heart Outcomes Prevention Evaluation (HOPE) Study Investigators, 2000. Effects of ramipril on cardiovascular and microvascular outcomes in people with diabetes mellitus: results of the HOPE study and MICRO-HOPE substudy. Lancet 355, 253–259.

Henriksen, E.J., Jacob, S., 1995. Effects of captopril on glucose transport activity in skeletal muscle of obese Zucker rats. Metabolism 44, 267–272.

Imanaka, T., Hayashi, H., Kishi, K., Wang, L., Ishii, K., Hazeki, O., Katada, T., Ebina, Y., 1998. Reconstitution of insulin signaling pathways in rat 3Y1 cells lacking insulin receptor and insulin receptor substrate-1. J. Biol. Chem. 273, 25347–25355.

Ishizuka, T., Miura, A., Kajita, K., Yamada, K., Wada, H., Itaya, S., Kanoh, Y., Ishizawa, M., Kimura, M., Yasuda, K., 1998. Alterations in insulin-induced postreceptor signaling in adipocytes of the Otsuka Long–Evans Tokushima fatty rat strain. J. Endocrinol. 156, 1–13.

Julius, S., Jamerson, K., 1994. Sympathetics, insulin resistance and coronary risk in hypertension: the “chicken-and-egg” question. J. Hypertens. 12, 495–502.

Kaplan, N.M., 1998. Obesity in hypertension: effects on prognosis and treatment. J. Hypertens. (Suppl. 16), S35–S37.

Kawano, K., Hirashima, T., Mori, S., Saitoh, Y., Kurosumi, M., Natori, T., 1992. Spontaneous long-term hyperglycemic rat with diabetic complications. Otsuka Long–Evans Tokushima Fatty (OLETF) strain. Diabetes 41, 1422–1428.

Kergoat, M., Portha, B., 1985. In vivo hepatic and peripheral insulin sensitivity in rats with non-insulin-dependent diabetes induced by streptozocin. Assessment with the insulin-glucose clamp technique. Diabetes 34, 1120–1126.

Kishi, K., Muromoto, N., Nakaya, Y., Miyata, I., Hagi, A., Hayashi, H., Ebina, Y., 1998. Bradykinin directly triggers GLUT4 translocation via an insulin-independent pathway. Diabetes 47, 550–558.

Manson, J.E., Colditz, G.A., Stampfer, M.J., Willett, W.C., Krolewski, A.S., Rosner, B., Arky, R.A., Speizer, F.E., Hennekens, C.H., 1991. A prospective study of maturity-onset diabetes mellitus and risk of coronary heart disease and stroke in women. Arch. Intern. Med. 151, 1141–1147.

Merrall, N.W., Plevin, R.J., Stokoe, D., Cohen, P., Nebreda, A.R., Gould, G.W., 1993. Mitogen-activated protein kinase (MAP kinase), MAP kinase kinase and c-Mos stimulate glucose transport in *Xenopus* oocytes. Biochem. J. 295, 351–355.

Moan, A., Hoieggen, A., Nordby, G., Eide, I.K., Kjeldsen, S.E., 1995. Effects of losartan on insulin sensitivity in severe hypertension: connections through sympathetic nervous system activity? J. Hum. Hypertens. 9 (Suppl. 5), S45–S50.

Moan, A., Hoieggen, A., Seljeflot, I., Risanger, T., Arnesen, H., Kjeldsen, S.E., 1996. The effect of angiotensin II receptor antagonism with losartan on glucose metabolism and insulin sensitivity. J. Hypertens. 14, 1093–1097.

National High Blood Pressure Education Program Working Group, 1994. National High Blood Pressure Education Program Working Group report on hypertension in diabetes. Hypertension 23, 145–160.

Nawano, M., Anai, M., Funaki, M., Kobayashi, H., Kanda, A., Fukushima, Y., Inukai, K., Ogihara, T., Sakoda, H., Onishi, Y., Kikuchi, M., Yazaki, Y., Oka, Y., Asano, T., 1999. Imidapril, an angiotensin-converting enzyme inhibitor, improves insulin sensitivity by enhancing signal transduction via insulin receptor substrate proteins and improving vascular resistance in the Zucker fatty rat. Metabolism 48, 1248–1255.

Reaven, G.M., 1991. Insulin resistance and compensatory hyperinsulinemia: role in hypertension, dyslipidemia, and coronary heart disease. Am. Heart. J. 121, 1283–1288.

Sowers, J.R., Epstein, M., 1995. Diabetes mellitus and associated hypertension, vascular disease, and nephropathy. Hypertension 26, 869–879.

Sweeney, G., Somwar, R., Ramlal, T., Volchuk, A., Ueyama, A., Klip, A., 1999. An inhibitor of p38 mitogen-activated protein kinase prevents insulin-stimulated glucose transport but not glucose transporter translocation in 3T3-L1 adipocytes and L6 myotubes. J. Biol. Chem. 274, 10071–10078.

Tomiyama, H., Kushiro, T., Abeta, H., Ishii, T., Takahashi, A., Furukawa, L., Asagami, T., Hino, T., Saito, F., Otsuka, Y. et al., 1994. Kinins contribute to the improvement of insulin sensitivity during treatment with angiotensin converting enzyme inhibitor. Hypertension 23, 450–455.

Tong, H., Chen, W., London, R.E., Murphy, E., Steenbergen, C., 2000. Preconditioning enhanced glucose uptake is mediated by p38 MAP kinase not by phosphatidylinositol 3-kinase. J. Biol. Chem. 275, 11981–11986.

Uusitupa, M.I., 1996. Early lifestyle intervention in patients with non-insulin-dependent diabetes mellitus and impaired glucose tolerance. Ann. Med. 28, 445–449.

Wang, L., Hayashi, H., Kishi, K., Huang, L., Hagi, A., Tamaoka, K., Hawkins, P.T., Ebina, Y., 2000. G_i-mediated translocation of GLUT4 is independent of p85/p110alpha and p110gamma phosphoinositide 3-kinases but might involve the activation of Akt kinase. Biochem. J. 345, 543–555.

Yagi, K., Kim, S., Wanibuchi, H., Yamashita, T., Yamamura, Y., Iwao, H., 1997. Characteristics of diabetes, blood pressure, and cardiac and renal complications in Otsuka Long–Evans Tokushima Fatty rats. Hypertension 29, 728–735.

Yoshizumi, M., Abe, J., Haendeler, J., Huang, Q., Berk, B.C., 2000. Src and Cas mediate JNK activation but not ERK1/2 and p38 kinases by reactive oxygen species. J. Biol. Chem. 275, 11706–11712.