Effects of Cilnidipine on Muscle Fiber Composition, Capillary Density and Muscle Blood Flow in Fructose-Fed Rats

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The aim of this study was to examine the roles of muscle fiber composition, capillary density and muscle blood flow in insulin resistance (IR) and the effect of cilnidipine, a calcium channel blocker in fructose-fed rats (FFR). Six-week-old male Sprague-Dawley rats were fed either normal rat chow or fructose-rich chow for 6 weeks. For the last 2 weeks, the rats were treated by gavage with a vehicle (Control and FFR groups) or with cilnidipine (FFR+Cil group). Blood pressure (BP) and insulin sensitivity were assessed in the sixth week. Muscle fiber composition, capillary density and blood flow in the soleus muscle were evaluated. BP of FFR was significantly higher than that of the controls. Cilnidipine significantly lowered BP in FFR. Insulin sensitivity was significantly lower in FFR than in the controls. Cilnidipine significantly improved IR in FFR. The composite ratio of type I fibers in the soleus muscle was significantly lower in FFR than in the controls, but that of type II fibers was significantly higher in FFR. Treatment with cilnidipine resulted in recovery of this ratio to that of the controls. Insulin sensitivity was found to be significantly correlated with the composite ratio of either type I fibers or type II fibers. There were no intergroup differences in capillary density. Muscle blood flow in the FFR+Cil group was higher than that in the Control or FFR groups. These results suggest that muscle fiber composition is linked to IR and that cilnidipine may improve IR in FFR either by modulating muscle fiber composition or by increasing muscle blood flow. (Hypertens Res 2001; 24: 565–572)

Key Words: insulin resistance, muscle fiber composition, capillary density, muscle blood flow, calcium channel blocker

Introduction

Recently, insulin resistance (IR) and hyperinsulinemia have been shown to be common findings in patients with essential hypertension, diabetes mellitus, or hyperlipidemia (1). These impairments of glucose metabolism are thought to be associated with each other and to play a role in the development of arteriosclerosis and cardiovascular diseases (2). IR has been reported in several animal models of hypertension—e.g., spontaneously hypertensive rats and fructose-induced hypertensive rats (3). In fructose-fed rats (FFR), it has been shown that a diet of fructose induces a decrease in glucose tolerance and a diet-induced elevation of blood pressure (4). Although it has been speculated that elevation of blood pressure is secondary to the development of IR and hyperinsulinemia, the precise mechanism of IR in FFR remains unclear.

The skeletal muscle is considered to be a major regulator of systemic insulin sensitivity. Euglycemic hyperinsulinemic glucose clamp studies have demonstrated that the skeletal muscle accounts for over 80% of glucose disposal under hyperinsulinemic conditions in humans. Several animal studies have demonstrated that substantial differences exist between muscle groups in insulin-mediated glucose uptake (IMGU),

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and that these differences may be related to muscle fiber composition (5). Moreover, muscles with the type I fibers are more insulin-sensitive than are those with type II fibers because of the difference in the densities of insulin receptors and in glucose transporter 4 (GLUT4) protein levels (6, 7). We previously reported that FFR show a significantly lower composite ratio of type I fibers in the soleus muscle than do Sprague-Dawley rats (8, 9). Thus, changes in muscle fiber composition may be one of the main mechanisms of IR in FFR.

Similarly, IMGU was found to be correlated with capillary density. The density of the capillary network of muscle has been found to be diminished both in patients with non-insulin dependent diabetes mellitus (NIDDM) (10) and obesity (11) as well as in experimental animal models (12, 13). It was suggested that the diffusion distance from capillary to muscle cell, or some associated biochemical change related to the diffusion distance, contributed to insulin resistance. Muscle blood flow may be associated with IMGU in skeletal muscle by alternation of insulin and glucose delivery (14). Insulin increases skeletal muscle blood flow in a dose-dependent fashion in lean normal subjects, but this effect is blunted in insulin-resistant obese individuals (15). In young men with borderline elevation of blood pressure, insulin sensitivity was found to be correlated with maximum forearm blood flow (16). Muscle blood flow might be one of the factors determining insulin sensitivity.

Regarding the effects of hypotension on insulin sensitivity, it has been shown that angiotensin-converting enzyme inhibitors (ACEIs), α-blockers, and long-acting calcium channel blockers improve insulin sensitivity in essential hypertensives. We have reported that calcium channel blockers improved IR in hypertensives (17) and in FFR (8). However, the precise mechanisms remain unknown. This study was designed to examine muscle fiber composition, capillary density and muscle blood flow, as well as to clarify the effect of a calcium channel blocker on insulin sensitivity and these factors in FFR.

Methods

General Protocol

Six-week-old male Sprague-Dawley rats (Charles River Japan Inc., Yokohama, Japan) were used for the experiments. The care of the animals was in strict accordance with the guiding principles of the Physiological Society of Japan. Before any manipulation, all rats were fed standard rat chow containing 60% vegetable starch, 5% fat and 24% protein (Oriental Yeast Co., Tokyo). They were maintained on a 12-h light/dark cycle and chow ad libitum. The rats were acclimated to handling before randomization, then divided into two groups at the start of study: those fed standard chow (control; n = 11) or those fed fructose-rich chow containing 60% fructose, 5% fat and 24% protein (#78463; Teklad, Madison, USA) (FFR; n = 18) for 6 weeks. The latter group of rats was treated either with 3 mg/kg/day of cilnidipine in 2.5% gum arabic solution (FFR + Cil; n = 7) or with a vehicle (2.5% gum arabic solution) (FFR; n = 11), and controls were treated with the same vehicle, by gavage, for the last 2 weeks. At the sixth week, systolic blood pressure (SBP) and insulin sensitivity were assessed in all conscious rats.

Blood Pressure and Heart Rate Measurement

SBP and heart rate (HR) were measured in all conscious rats using the indirect tail-cuff method (8). The rats were preconditioned to the experimental procedure before actual measurements were conducted. The equipment included a blood pressure sensor/cuff, a blood pressure amplifier, and digital recorder (Natsume Seisakusho Co., Ltd., Tokyo). The averages of six such recordings were taken as the individual SBP and HR. This method correlates highly with direct measurements.

Euglycemic Hyperinsulinemic Glucose Clamp Technique

At the end of the treatment period, rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.). The left common carotid artery and the left jugular vein were exposed and then cannulated with a polyethylene tube (PE50; Becton Dickinson and Co., Sparks, USA) for collecting blood samples and for administration of the infusate. After overnight fasting (approximately 12 h), each rat was placed in a foam plastic jacket, which allowed movement of all four limbs and forward vision. At the start of the clamp, fasting blood glucose measurements were obtained, and the initial load of insulin (25 μU/kg of Humulin R, U-40; Shionogi Pharmaceutical Co., Osaka, Japan) was infused by a bolus, followed by an infusion of insulin at a rate of 4 μU/kg/min for 150 min. During the clamp, 12.5% glucose solution was infused as needed to maintain blood glucose at the preinfusion level. Ten microliters of arterial blood was sampled at 7-min intervals for determination of blood glucose. The average of the rate of glucose infusion for the last 35 min was taken as the index of insulin sensitivity (M value) of each rat (8). Figure 1 shows the algorithm used for the glucose clamp study (9).

Determination of Muscle Fiber Composition and Capillary Density

Under anesthesia with sodium pentobarbital (50 mg/kg i.p.), the soleus muscles of each group were dissected out and immediately frozen in liquid nitrogen. Ten-micrometer-thick sections sliced by a microtome were stained with 4 mmol/l adenosine triphosphatase and 18 mmol/l CaCl2 at pH 9.5 for 45 min at room temperature after preincubation at pH 10.4 or 4.3 (18). Muscle fiber composition was then determined under a low-powered microscope. Only type I fibers are characterized by dark staining after preincubation at pH 4.3.
Type II fibers are sensitive to dark stain after preincubation at pH 10.4. Two investigators individually counted a minimum of 400 fibers after coding preparations to minimize individual bias. For estimation of capillary density, ten-μm-thick sections of soleus muscle sliced by a microtome were stained with Schiff’s reagent for 30 min at room temperature after fixation in Carnoy’s fixative for 10 min and digestion of glycogen with 1% alpha-amylase at 37°C for 30 min and oxidation in 1% periodic acid for 10 min. Capillary density was then determined under a low-powered microscope (19). Two investigators individually counted a minimum of 800 fibers after coding preparations to minimize individual bias.

Determination of Muscle Blood Flow

We prepared another series of rats (Control: n = 9; FFR: n = 8; FFR+Cil: n = 9). Under anesthesia with sodium pentobarbital (50 mg/kg i.p.), the right common carotid artery and the left femoral artery were exposed and then cannulated with PE50 for administration of the infusate and blood reference sampling. A line via the right carotid artery was inserted into the left ventricle by observing the characteristic left ventricular waveform. Radiolabelled microspheres (NEN Life Science Products Inc., Boston, USA; 15 ± 0.2 μm in diameter, labeled with 51Cr) were utilized to estimate soleus muscle blood flow according to the reference sample technique as adapted for use in the rat. Prior to injection, the microspheres were suspended in 0.9% sodium chloride and 0.01% Tween 80. Following ultrasonification and mixing on a vortex mixer, the microspheres (approximately 150,000 spheres in 0.5 ml) were drawn into a 1 ml heparinized plastic disposal syringe through an 18-gauge needle. From the femoral arterial catheter, reference blood flow was withdrawn at a rate of 0.204 ml/min with a Harvard withdrawal pump (Harvard Apparatus Inc., South Natick, USA) into a 5-ml heparinized disposal syringe. Thirty seconds after the beginning of withdrawal, 0.5 ml of the microsphere suspension was injected into the left ventricle via the right carotid arterial catheter over a period of 15 s. The suspension was then flushed through the left ventricle over a period of 15 s. The reference blood flow sample withdrawal was continued for 4 min. Two minutes after the end of withdrawal, a right soleus muscle was dissected and placed into a preweighed vial for analysis of radioactivity. The vial was reweighed and packed in a gamma counter for measurement of radioactivity (20). With the data obtained from the sphere procedures, muscle blood flow was calculated as follows:

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\text{Muscle blood flow (ml/g/min) = 0.204} \times \text{Soleus muscle activity/Reference blood flow activity/Soleus muscle weight.}
\]

Biochemical Measurements

Blood glucose levels were measured by the glucose oxidase method in an E-xact 2A glucose analyzer (MediSense, Inc., Waltham, USA).

Statistical Analysis

All data are expressed as the means ± SEM. Data were statistically analyzed with one-way analysis of variance (ANOVA) followed by the Fisher’s PLSD test for multiple comparisons. Regression analyses were used to compare the relationship between M values and muscle fiber composition. Values of p < 0.05 were considered to indicate statistical significance.
Table 1. Characteristics of Each Group at the Age of 12 Weeks

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>FFR</th>
<th>FFR+Cil</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (g)</td>
<td>369±8</td>
<td>336±5 *</td>
<td>335±11 *</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>142±3</td>
<td>157±3 **</td>
<td>146±4</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>345±7</td>
<td>393±10 *</td>
<td>368±10</td>
</tr>
<tr>
<td>FBS (mmol/l)</td>
<td>5.4±0.1</td>
<td>5.5±0.1</td>
<td>5.1±0.1</td>
</tr>
<tr>
<td>Ht (%)</td>
<td>43.1±0.4</td>
<td>42.3±0.5</td>
<td>43.9±0.7</td>
</tr>
</tbody>
</table>

FFR, fructose-fed rats treated with vehicle; FFR+Cil, fructose-fed rats treated with cilnidipine; BW, body weight; SBP, systolic blood pressure; HR, heart rate; FBS, fasting blood sugar; Ht, hematocrit. Values are the means±SEM. *p<0.01 vs. Control; **p<0.05 vs. FFR+Cil.

Glucose Clamp and Muscle Fiber Composition

Figure 2 shows the time course of the glucose clamp study in each group. Steady-state blood glucose levels during the glucose clamp were similar in the 3 experimental groups. The M value was significantly lower in FFR (10.4±0.9 mg/kg/min, p<0.01) than that in the controls (15.3±0.6 mg/kg/min). Cilnidipine significantly improved insulin resistance in FFR (13.0±0.7 mg/kg/min, p<0.05). The composite ratio of type I fibers in the soleus muscle was significantly lower in FFR (75.0±1.8%, p<0.05) than in the controls (81.4±1.7%). Treatment with cilnidipine for 2 weeks led to a recovery of the composite ratio of type I fibers in the soleus muscle of FFR (81.7±1.3%, p<0.05 compared with FFR) to the same level as that of the controls (Fig. 3A). The composite ratio of type II fibers in the soleus muscle was significantly higher in FFR (25.0±1.8%, p<0.05) than in the controls (18.6±1.7%). Treatment with cilnidipine for 2 weeks led to a recovery of the composite ratio of type II fibers in the soleus muscle of FFR (18.3±1.7%, p<0.05 vs. FFR) to the control level (Fig. 3B). The M value showed a significant positive correlation with the composite ratio of type I fibers and a significant negative correlation with the composite ratio of type II fibers (Fig. 4).

Capillary Density and Muscle Blood Flow

There were no significant intergroup differences in capillaries/fiber or capillaries/area (Fig. 5). There was no significant difference between muscle blood flow in the Control and FFR groups (0.24±0.2 vs. 0.21±0.2 ml/g/min, respectively) (Fig. 6). However, muscle blood flow in the FFR+Cil group (0.52±0.9 ml/g/min, p<0.01) was obviously higher than that in the Control or FFR group.

Discussion

This study confirmed previously reported results showing that feeding healthy rats fructose-rich chow results in IR and
hypertension. The composite ratio of type I fibers was lower in FFR than in the controls, and inversely, the composite ratio of type II fibers was higher in the FFR than in Control rats. Treatment with cilnidipine resulted in recovery of these ratios to the Control value. The M value was significantly correlated with the composite ratios of both fiber types. Although there was no significant difference in capillary density or muscle blood flow between the Control and FFR groups, muscle blood flow in the FFR + Cil group became higher than that in the Control or FFR group. These results indicated that muscle fiber composition plays a role in modulation of IR in FFR and that cilnidipine improved IR by changing the muscle fiber composition and increasing muscle blood flow. Several studies have shown the effects of calcium channel blockers on IR in essential hypertensives or insulin-resistant animal models. However, the effects of calcium channel blockers remain controversial, and possible mechanisms by which calcium channel blockers improve an insulin-resistant state have only been described in a few reports. To the best of our knowledge, the present study is the first to investigate muscle fiber composition, capillary density and muscle blood flow in FFR after treatment with cilni-

dipine.

It is not clear why BW was lower in the FFR and FFR + Cil groups than in the control group. We did not evaluate food consumption in each group. However, Mooradian et al. (21) reported that the food consumption of FFR was lower than that of the rats fed standard chow after the tenth day of their experiment, although there was no difference in BW between the two groups. Reaven (3) reported that the BW of FFR was slightly, but not significantly, lower than that of rats fed standard chow after the third week of the experiment. These findings suggest that the difference in food consumption between the Control and FFR groups might have affected BW in the present study. It is well known that insulin sensitivity is negatively correlated with BW. Thus, lower BW did not play an important role of IR in FFR.

Type I fibers are slow twitch fibers, have an abundance of mitochondria and work oxidatively, while type II fibers are fast twitch fibers, have fewer mitochondria and use more glycolytic pathways (5). The former fiber type is more insulin-sensitive than the latter. The latter is further subclassified as type IIA and type IIB fibers. Type IIB fibers are less insulin-sensitive than are type IIA fibers. Although muscle fiber
Fig. 5. Comparisons of capillary density (capillaries/fiber (Cap/Fiber) (A) and capillaries/area (Cap/Area) (B)) in the three groups. FFR: fructose-fed rats treated with vehicle, FFR+Cil: fructose-fed rats treated with cilnidipine. Columns and bars are the means±SEM.

sympathetic nerve activation might contribute to the change in muscle fiber composition. Considering the results of these studies, it is possible that compensatory hyperinsulinemia itself and sympathetic nervous activation induced by hyperinsulinemia may also cause a change from type I to type II fibers, resulting in further IR.

Nowycky et al. (28) demonstrated the existence of three types of voltage-dependent calcium channels, L-, N-, and T-types, on the basis of electrophysiological characterization. In particular, the N-type calcium channel is expressed on sympathetic nerve endings and regulates the cardiovascular functions via release of catecholamine. Cilnidipine is a new and unique 1,4-dihydropyridine calcium blocker that has both L-type and N-type voltage-dependent calcium channel blocking actions, and it acts on vasodilatation and suppression of sympathetic nerve activity (29). Cilnidipine should be more beneficial than other calcium channel blockers for the treatment of insulin-resistant hypertension. Our previous study (8) using benidipine showed no changes of HR in FFR (8), however, cilnidipine lowered HR in FFR irrespective of a similar reduction of blood pressure. These results indicate that cilnidipine had some effect on the blocking of N-type channels, whereas it had little effect on IR.

There have been several reports on the correlation between insulin resistance and reduction in capillary density. In the present study, we expected that FFR would have a lower capillary density than the controls, but no difference in capillary density was found. In NIDDM (10) and obesity (11), reduction in capillary density was found in the change of muscle fiber composition from type I to type II fibers, and particularly to type Iib fibers. In humans, type I fibers and type Ila fibers have high capillary densities, while type Iib fibers have a low capillary density (19). It is thought that an increase of type Iib fibers contributes to the reduction in capillary density. Although we did not subclassify type II fibers in the present study, our previous study showed that there was a decrease in the percentage of type I fibers and an increase in...
the percentage of type IIA fibers in FFR and that treatment with benidipine resulted in recovery of the composite ratios of type I and type IIA fibers to the control level (8). There were no significant intergroup differences in type IIB fibers in the present study. Therefore, if the change in muscle fiber composition is the same as that by benidipine, it is rather difficult to find any difference in capillary density based on only the change between type IIA and type I fibers. If we had evaluated the extensor digitorum longus, which has many type IIB fibers, we might have found a significant difference in capillary density between the Control and FFR groups. On the other hand, in a previous study the capillary density in the soleus muscle of deoxycorticosterone acetate-salt hypertensive rats was lower than that in the control group after 14 weeks of treatment, although no difference in capillary densities had been observed after 7 weeks of treatment (13). It is therefore thought that the experimental periods in this previous study were not sufficient to detect any changes in capillary density in the soleus in FFR.

Although there was no difference in capillary densities between the FFR and FFR+Cil groups in the present study, cilnidipine increased the skeletal muscle blood flow in FFR. Baron et al. (14) reported that the percent increment of muscle blood flow is positively correlated to the rate of IMGU. In a study on the Zucker fatty rat, the ACEI imidapril contributed to improvement of IR by increasing muscle blood flow and acting on the insulin-signaling pathway (30). Muscle blood flow may alter glucose uptake in skeletal muscle by increasing glucose and insulin delivery to skeletal muscle. A cilnidipine-induced increase in the blood supply to skeletal muscle may thus improve the IR in FFR.

In conclusion, the results of this study suggest that the fiber composition of skeletal muscle is linked to IR and that cilnidipine, a calcium channel blocker, may improve IR in FFR either by modulating muscle fiber composition or by increasing muscle blood flow. These results indicate that cilnidipine may have the potential to improve IR as well as to lower blood pressure in patients with insulin-resistant hypertension. Since insulin-resistant hypertension may lead to development of multiple organ damage, cilnidipine might be an effective hypotonic for treating insulin-resistant hypertension and accompanying complications, such as arteriosclerosis and cardiovascular disease.

References


