Altered Pressure-Natriuresis in Obese Zucker Rats

Keiji Fujiwara, Koichi Hayashi, Hiroto Matsuda, Eiji Kubota, Masanori Honda, Yuri Ozawa, Takao Saruta

Abstract—It has not been examined whether the pressure-natriuresis response is altered in the insulin-resistant condition. Furthermore, despite an important role of nitric oxide (NO) in modulating pressure-natriuresis, no investigations have been conducted assessing the renal interstitial NO production in insulin resistance. The present study examined whether pressure-natriuresis was altered in insulin-resistant obese Zucker rats (OZ) and assessed the cortical and medullary nitrate/nitrite (NOx) levels with the use of the renal microdialysis technique. In OZ, serum insulin/glucose ratio (23.0±4.0×10⁻⁸, n = 9) and blood pressure (119±3 mm Hg) were greater than those in lean Zucker rats (LZ; 7.0±1.9×10⁻⁸ and 103±4 mm Hg, n = 9). The pressure-natriuresis curve in OZ was shifted to higher renal perfusion pressure (RPP), and the slope was blunted compared with that in LZ (0.073±0.015 vs 0.217±0.047 μEq/min kidney weight/mm Hg, P < 0.05). The basal renal NOx level was reduced in OZ (cortex, 4.032±0.331 μmol/L; medulla, 4.329±0.515 μmol/L) compared with that in LZ (cortex, 7.315±1.102 μmol/L; medulla: 7.698±0.964 μmol/L). Furthermore, elevating RPP increased the medullary NOx in LZ, but this pressure-induced response was lost in OZ. Four-week treatment with troglitazone, an insulin-sensitizing agent, improved hyperinsulinemia, systemic hypertension, and basal renal NOx levels (cortex, 5.639±0.286 μmol/L; medulla, 5.978±0.284 μmol/L), and partially ameliorated the pressure-natriuresis curves; the slope of pressure-natriuresis curves and elevated RPP-induced NOx, however, were not corrected. In conclusion, our study suggests that insulin resistance is closely associated with abnormal pressure-natriuresis and hypertension. These deranged renal responses to insulin resistance are most likely attributed to impaired medullary NO production within the medulla. (Hypertension. 1999;33:1470-1475.)

Key Words: natriuresis ■ nitric oxide ■ insulin resistance ■ hypertension ■ troglitazone ■ microdialysis ■ obesity

A growing body of evidence has been accumulated demonstrating that insulin resistance is an important risk factor to the development of cardiovascular diseases, including hypertension.1–3 Although the pathophysiology of hypertension in insulin resistance remains fully undetermined, several investigations suggest that the kidney plays an important role in the development of hypertension.4 Thus renal sodium homeostatic mechanisms are reported to be impaired in obesity, in which insulin resistance conceivably develops.5 Furthermore, we have recently demonstrated that the pressure-natriuresis relation is impaired in Wistar fatty rats, manifesting obesity, hyperinsulinemia, and hyperglycemia.6 Thus balanced sodium homeostasis is attained only at a higher renal perfusion pressure (RPP) in these obese rats than in normal littermates. The altered relation between RPP and renal sodium excretion is characteristic of several models of experimental hypertension, including spontaneously hypertensive rats and Dahl salt-sensitive rats7,8 and is generally accepted to be responsible for the development of hypertension in these rats. In Wistar fatty rats, however, hyperglycemia may also modify the renal sodium excretory function6 and may thus confound the renal effect of insulin resistance per se. The relation between pressure-natriuresis and insulin sensitivity therefore has not been delineated.

Recently, much attention has been focussed on the mechanisms of pressure-natriuresis. Several investigations have been conducted examining the role of nitric oxide (NO) in pressure-natriuresis in hypertensive animals.7–9 The infusion of L-arginine methyl ester into the renal medulla markedly blunted pressure-natriuresis.10 Furthermore, L-arginine corrected the impaired pressure-natriuresis when administered in hypertensive animals.9 These studies suggest an important role of NO in the pressure-natriuresis response, and impaired sodium excretion is closely associated with altered NO activity within the medulla in hypertensive animals. Nevertheless, no investigations have been performed to clarify the effect of insulin resistance on renal interstitial NO release in obese animals.

In the present study, we investigated whether pressure-natriuresis was altered in insulin-resistant obese Zucker rats (OZ). Furthermore, the role of renal medullary and cortical interstitial NO in mediating pressure-natriuresis was assessed by evaluating the renal interstitial nitrate/nitrite (NOx) concentrations in these rats. Finally, to elucidate whether the

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correction of insulin resistance altered pressure-natriuresis, the effect of troglitazone, a novel thiazolidine-type insulin-sensitizing agent,\textsuperscript{11} on pressure-natriuresis response was examined.

**Methods**

**Animals**

All experimental protocols were conducted according to the guidelines of the Animal Care Committee of Keio University. Eighteen 4-week-old male OZ rats and their controls (lean Zucker, LZ) were used. The rats were divided into the following groups: (1) LZ (n=9); (2) LZ+troglitazone (n=9); (3) OZ (n=9); and (4) OZ+troglitazone (n=9). They were all prohibited from feeding 12 hours before the surgical preparation. They were placed in metabolic cages and were fed a standard rat chow (15% protein, 5% fat, 58% carbohydrate).

**Pressure-Natriuresis**

Animal preparation was performed as detailed previously.\textsuperscript{6–10} In brief, the right kidney and the right adrenal gland were removed 7 days before the experiment. For renal perfusion study, animals were anesthetized with sodium pentobarbital (50 mg/kg IP, Abbott) and tracheostomized to facilitate respiration. The left carotid and femoral arteries were cannulated (PE50, Becton Dickinson Co) to measure arterial blood pressure (BP) with the use of a pressure transducer (TP400T, Nihon Koden). The left external jugular vein was cannulated (PE50) for the infusion of saline containing a hormone cocktail (TP400T, Nihon Koden). The left external jugular vein was cannulated (PE50) for the infusion of saline containing a hormone cocktail described previously.\textsuperscript{6–10} With laparotomy, the left adrenal gland was removed and the left kidney was denervated with a 10% phenol solution in ethanol. An additional cannula (PE10) was placed in the left ureter for the collection of urine.

An adjustable clamp was placed on the aorta below left renal artery. Silk ligatures were placed loosely around the mesenteric and celiac arteries. RPP was altered by adjusting the resistance of these vessel segments.

**Microdialysis**

For determination of renal interstitial NOx concentrations, we used a renal microdialysis technique.\textsuperscript{12,13} This method has been reported to detect angiotensin-converting enzyme inhibitor–induced increases in medullary and cortical NOx contents.\textsuperscript{13} A microdialysis tube (0.5-mm diameter and 10-kDa transmembrane diffusion cutoff; Eicom) was inserted into the cortex and medulla at a depth of 1 mm and 5 mm, respectively, from the renal surface. The microdialysis tube was perfused with lactated Ringer’s solution (147 mEq/L Na, 4 mEq/L K, 5 mEq/L Ca, 156 mEq/L Cl) at 2 μL/min. At this rate, in vitro recovery was 78±3% for nitrate and 70±4% for nitrite. A 120-minute stabilization period was allowed before the experiments. The effluent was stored at −20°C until NOx measurements by Griess reaction.\textsuperscript{14}

**Experimental Protocols**

After a 120-minute equilibration period, the baseline BP was measured. Urine was collected during two 25-minute periods to measure urinary excretions of sodium, insulin, and para-aminohippuric acid (PAH). Then, the mesenteric and celiac arteries were occluded to increase RPP. After 15 minutes, two 25-minute renal clearance studies were conducted. Then, RPP was further elevated by tightening the clamp below renal artery. At the midpoint of 2 clearance periods at each RPP, 1 mL of arterial blood was drawn, and an equal volume of blood from the similarly treated littermate was replaced. Values obtained from the 2 clearance periods were averaged.

Sodium concentrations were measured with a flame photometer. Insulin and PAH concentrations were measured by standard photometry. Glomerular filtration rate (GFR) and renal plasma flow (RPF) were calculated as the ratio of the urine per plasma concentration of insulin and PAH multiplied by urine flow rate.

**Statistics**

Results are expressed as mean±SEM. Statistical analysis was evaluated by 2-way ANOVA, followed by multiple comparison post hoc test. A value of $P<0.05$ was considered statistically significant.

**Results**

**Body Weight, BP, and Laboratory Data**

Body weight and serum glucose concentration did not differ among groups (Table 1). OZ rats manifested elevated concentrations of serum insulin ($P<0.01$) and serum insulin-to-glucose ratio ($P<0.05$), a marker of insulin resistance.\textsuperscript{15} These elevated levels were markedly decreased by the treatment with troglitazone to those observed in LZ with or without troglitazone.

The basal BP in OZ rats (119±3 mm Hg, n=9) was higher than that in LZ rats (103±4 mm Hg, n=9, $P<0.01$) (Table 1). The treatment with troglitazone reduced the BP in OZ (OZ+troglitazone, 106±2 mm Hg [$P<0.05$, n=9] vs OZ) but had no effect on the BP in LZ (LZ+troglitazone, 99±5 mm Hg, n=9).

**Renal Function and Pressure-Natriuresis**

Basal GFR or RPF did not differ among groups (Table 2). In all groups, GFR was maintained constant during alterations in RPP. In response to elevated RPP, RPF increased in OZ (from 1.94±0.07 to 2.25±0.05 mL/min per gram of kidney weight, $P<0.01$) but was not altered in LZ, LZ+troglitazone, or OZ+troglitazone.

Elevated RPP markedly increased urine flow by 226±32% in LZ and by 196±37% in LZ+troglitazone. In OZ, incre-
ments in urine flow were diminished (123±10%, \( P<0.01 \)), and troglitazone partially improved the pressure-induced increases in urine flow (178±7%).

Figure 1 illustrates the pressure-natriuresis relation in Zucker rats. When RPP was elevated from baseline pressure to the maximal RPP examined, sodium excretion was prominently increased by 238±11% in LZ (from 3.06±0.65 to 11.17±2.14 \( \mu \text{Eq/min per gram of kidney weight/mm Hg} \), \( P<0.01 \)) and by 266±52% in LZ+troglitazone (from 3.34±0.51 to 12.03±1.75 \( \mu \text{Eq/min per gram of kidney weight/mm Hg} \), \( P<0.01 \)); no difference was noted between LZ and LZ+troglitazone. In contrast, the pressure-natriuretic response was markedly blunted in OZ and troglitazone-treated OZ, with only 103±36% (from 2.85±0.47 to 5.89±1.14 \( \mu \text{Eq/min per gram of kidney weight} \), \( P<0.01 \)) and 173±18% increments in sodium excretion (from 3.11±0.62 to 8.42±0.70 \( \mu \text{Eq/min per gram of kidney weight} \), \( P<0.01 \)), respectively.

Table 2. Effects of Renal Perfusion Pressure on Renal Function in Zucker Rats

<table>
<thead>
<tr>
<th>Renal Pressure, mm Hg</th>
<th>Renal Plasma Flow, mL/min/g k wt</th>
<th>Glomerular Filtration Rate, mL/min/g k wt</th>
<th>Urine Flow Rate, ( \mu \text{L/min/g k wt} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lean (n=7)</td>
<td>103±3</td>
<td>1.95±0.13</td>
<td>48±4</td>
</tr>
<tr>
<td>121±3</td>
<td>1.98±0.01</td>
<td>0.99±0.05</td>
<td>92±2†</td>
</tr>
<tr>
<td>140±1</td>
<td>2.17±0.12</td>
<td>0.97±0.13</td>
<td>148±8†</td>
</tr>
<tr>
<td>Lean+troglitazone (n=7)</td>
<td>99±2</td>
<td>1.87±0.04</td>
<td>52±7</td>
</tr>
<tr>
<td>118±2</td>
<td>1.98±0.04</td>
<td>1.02±0.03</td>
<td>108±24*</td>
</tr>
<tr>
<td>135±2</td>
<td>2.03±0.05</td>
<td>0.98±0.22</td>
<td>157±29†</td>
</tr>
<tr>
<td>Obese (n=7)</td>
<td>119±3</td>
<td>1.94±0.07</td>
<td>34±5</td>
</tr>
<tr>
<td>136±3</td>
<td>2.08±0.09</td>
<td>1.06±0.04</td>
<td>56±9†</td>
</tr>
<tr>
<td>160±3</td>
<td>2.25±0.05†</td>
<td>1.10±0.07</td>
<td>80±9†</td>
</tr>
<tr>
<td>Obese+troglitazone (n=7)</td>
<td>106±1</td>
<td>1.82±0.07</td>
<td>37±8</td>
</tr>
<tr>
<td>131±2</td>
<td>1.91±0.15</td>
<td>0.96±0.08</td>
<td>89±10†</td>
</tr>
<tr>
<td>152±4</td>
<td>1.98±0.17</td>
<td>0.95±0.03</td>
<td>100±12†</td>
</tr>
</tbody>
</table>

Lean indicates lean Zucker rats; Obese, obese Zucker rats; g k wt, gram kidney weight. Values are mean±SEM. *\( P<0.05 \) vs baseline. †\( P<0.01 \) vs baseline.

The slope of the relations between RPP and urinary sodium excretion was significantly less in OZ than in LZ (OZ, 0.073±0.015 vs LZ, 0.241±0.015 \( \mu \text{Eq/min per gram of kidney weight/mm Hg} \), \( P<0.05 \)). The treatment with troglitazone had no effect on the slope in LZ (LZ+troglitazone; 0.217±0.047 \( \mu \text{Eq/min per gram of kidney weight/mm Hg} \), \( P>0.05 \) vs LZ). In troglitazone-treated OZ, the slope of the pressure-natriuresis curve (0.113±0.008 \( \mu \text{Eq/min per gram of kidney weight/mm Hg} \)) did not differ from that in OZ, but the pressure-natriuresis curve was shifted to lower RPP.

Renal Cortical and Medullary NOx Levels

The renal cortical NOx levels at the baseline RPP were markedly diminished in OZ rats (4.032±0.331 \( \mu \text{mol/L} \), \( n=7 \)) compared with those in LZ (7.315±1.102 \( \mu \text{mol/L} \), \( P<0.01 \), \( n=7 \)) (Figure 2). Treatment with troglitazone restored, albeit partially, the cortical NOx levels (ie, LZ+troglitazone; 5.639±0.286 \( \mu \text{mol/L} \), \( n=7 \), \( P<0.01 \)) in OZ rats, whereas no additional effect was observed in LZ. Elevating RPP had no effect on cortical NOx contents in either group of rats.

Similarly, basal medullary NOx levels in OZ rats were decreased [4.329±0.515 \( \mu \text{mol/L} \), \( n=7 \)] vs 7.698±0.964 \( \mu \text{mol/L} \), \( n=7 \) for LZ, \( P<0.01 \) and were partially ameliorated by troglitazone (OZ+troglitazone; 5.978±0.284 \( \mu \text{mol/L} \), \( P<0.01 \), \( n=7 \)). In LZ, medullary NOx increased as RPP was elevated (from 7.698±0.964 to 10.156±1.275 \( \mu \text{mol/L} \), \( P<0.05 \), \( n=7 \)), and the treatment with troglitazone had no additional effect on RPP-induced increases in medullary NOx (LZ+troglitazone; from 8.116±0.847 to 10.505±0.579 \( \mu \text{mol/L} \), \( P<0.05 \), \( n=7 \)). Elevating RPP, however, failed to increase medullary NOx content in OZ (from 4.329±0.515 to 4.358±0.429 \( \mu \text{mol/L} \), \( n=7 \)) or OZ+troglitazone (from 5.978±0.284 to 6.346±0.264 \( \mu \text{mol/L} \), \( n=7 \)).

Discussion

A large number of investigations suggest that insulin resistance causes systemic hypertension.1–3 Although several lines
of investigations have reported that the kidney is responsible for the development of hypertension in the insulin-resistant condition, the precise mechanisms of insulin resistance–associated hypertension remain undetermined. Recently, NO has been established to participate importantly in the control of renal function as a factor determining both microvascular tone and tubular function, and the derangement of NO production/action has been proposed as a contributory factor to the development of hypertension in several forms of hypertension. It has not been examined, however, whether insulin resistance alters the pressure-natriuresis response. Furthermore, the role of intrarenal NO in pressure-natriuresis has not been assessed in insulin resistance.

The present study has demonstrated that OZ rats manifest not only metabolic abnormality associated with insulin resistance but also hemodynamic derangement, including systemic hypertension and impaired pressure-natriuresis. Furthermore, these alterations were reversed by troglitazone. Since the ratio of serum insulin to glucose, a marker of insulin sensitivity, parallels the systemic and renal hemodynamic changes in OZ rats, it reasonably can be speculated that these alterations are a consequence of impaired insulin sensitivity in this rat strain. Although the blood glucose levels appear higher in all groups of rats, an infusion of a hormone cocktail including catecholamine may acutely affect the blood glucose and its response to troglitazone. Of note, we previously found impaired pressure-natriuresis responses in Wistar fatty rats. These rats, however, manifested marked hyperglycemia and glucosuria, which may cause osmotic diuresis and thus affect tubular sodium excretion. The present observation that OZ and LZ rats exhibit similar blood glucose levels rather favors the possibility that insulin resistance per se participates importantly in abnormal pressure-natriuresis and hypertension and is in good accordance with the formulation that insulin resistance is importantly associated with systemic hypertension. A recent report by Abe et al demonstrating the development of insulin resistance and hypertension in insulin receptor substrate-1–deficient mice is also consistent with this formulation.

Traditionally, obesity is reported to exhibit salt-sensitive hypertension. It remains a matter of controversy, however, how insulin resistance is associated with impaired natriuresis. In the present study, we have demonstrated the impaired pressure-natriuresis in OZ rats. Thus the slope of the pressure-natriuresis curve is diminished, a finding similar to that in other hypertensive animals, including Dahl salt-sensitive rats, in which impaired pressure-natriuresis plays a crucial role in initiating or maintaining hypertension. In this regard, Kimura et al found that the blunted slope of the pressure-natriuresis curve was associated with the impaired tubular sodium excretion or the decreased glomerular filtration coefficient. It is anticipated therefore that OZ rats manifest salt sensitivity and subsequent hypertension. Furthermore, the blunted pressure-natriuresis in OZ rats despite nearly the same GFR in OZ and LZ rats should be attributed to the altered tubular sodium handling. Of note, it has recently been reported that OZ rats possess a fa/fa gene that is closely linked to leptin receptor mutation. Since leptin is demonstrated to exert natriuretic action, it may be that the absence of leptin action within the kidney impairs the renal sodium excretion and thus causes hypertension in OZ rats. Although it remains undetermined whether leptin actually plays a substantial role in modulating renal sodium excretion under physiological conditions, it is intriguing to hypothesize the renal effect of leptin in the pathogenesis of hypertension.

Recent studies have been conducted on the mechanisms whereby increased RPP inhibits renal tubular sodium absorption. Cowley et al demonstrated that the elevation of renal interstitial hydrostatic pressure (RIHP) was essential to intact pressure-natriuresis response, and subsequent studies have reported that papillary hemodynamics play an important role in the regulation of RIHP. Much attention has been focused on the role of NO in mediating pressure-natriuresis. Nakamura et al showed that the NO blockade reduced RIHP and impaired pressure-natriuresis. Furthermore, the present study demonstrates that the pressure-natriuresis response is impaired in OZ rats in which medullary NOx levels are reduced (Figure 2). In concert, available evidence suggests that renal medullary NO participates in the transmission of RPP to RIHP and the subsequent pressure-natriuresis. Of interest, Majid et

![Diagram](http://hyper.ahajournals.org/Downloadedfrom)
have recently demonstrated RPP-dependent alterations in renal cortical and medullary NO activity, using an NO-selective microelectrode. In the present study, we found that elevating RPP increased medullary NOx contents in LZ, but this response was lost in OZ. Furthermore, troglitazone failed to restore the medullary NOx responses or only partially corrected the pressure-natriuresis in OZ rats. Although the source of renal interstitial NOx remains undetermined, the parallel responses of medullary NO activity and NOx to elevated RPP suggest that at least a part of medullary NOx reflects actual NO within the kidney. Furthermore, the reduced basal NOx and impaired NOx responses to elevated RPP may be responsible for the blunted pressure-natriuresis in OZ rats. Thus it is intriguing to assume that the integrity of pressure-natriuresis requires an intact RPP-induced NO activity within the medulla. Of note, Zou and Cowley reported that renal interstitial NO release is greater in the medulla than in the cortex, whereas the present study indicated similar NO release in these zones. These discrepant observations may be related to the differences in the technique used; Zou and Cowley measured the NO levels by the hemoglobin trapping technique, whereas we used the Griess reaction.

Accumulating evidence suggests an effect of insulin resistance on vascular NO activity. Insulin is reported to cause vasodilation by enhancing NO release in several vascular beds, including the renal and forearm vessels. Furthermore, Walker et al found that insulin-mediated dilation of the mesenteric artery was diminished in OZ rats. We have recently demonstrated that insulin-induced dilation of the afferent arteriole is mediated by NO in the isolated perfused hydrenephrotic kidney, and this insulin-induced vasodilation is impaired in OZ rats. With the use of a renal microdialysis technique, the present study demonstrates that the renal NOx is markedly diminished in insulin-resistant OZ rats compared with that in LZ rats. To the extent that the medullary NO plays an important role in pressure-natriuresis, the diminished medullary NO production may contribute to the blunted pressure-natriuresis in OZ rats. In concert, these observations lend support to the contention that the insulin resistance is associated with impaired NO production, which could contribute to the development of hypertension. Of note, troglitazone completely corrected the serum insulin level and hypertension but partially ameliorated the renal interstitial NOx or pressure-natriuresis. Thus the reduced renal NO activity in OZ rats is not totally attributable to insulin resistance but may also be related to a genetic background of this rat strain. Alternatively, normalization of blood pressure by troglitazone may be due to direct inhibition of L-type calcium channels of the vascular smooth muscle or modulation of vascular remodeling, both of which may lead to improvement in pressure-natriuresis. Further studies are required to clarify these issues.

In conclusion, the present study demonstrates that obese Zucker rats, characterized by insulin resistance, exhibit impaired pressure-natriuresis and systemic hypertension. Furthermore, correction of insulin sensitivity by troglitazone improves, albeit partially, the blunted pressure-natriuresis response and renal interstitial NOx levels. Thus insulin sensitivity may constitute an important determinant of systemic hypertension and may also contribute at least in part to the integrity of pressure-natriuresis and renal NO production.

References


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