Antihypertensive Effect of Pioglitazone Is Not Invariably Associated With Increased Insulin Sensitivity

Hong Yan Zhang, Sreenivas R. Reddy, Theodore A. Kotchen

Abstract Hypertension is often associated with insulin resistance, and several chemically diverse agents that increase insulin sensitivity attenuate the development of experimental hypertension. We undertook the present study to determine whether attenuation of hypertension by pioglitazone, a thiazolidinedione derivative that increases insulin sensitivity without increasing insulin secretion, is specifically related to its effect on insulin-mediated glucose uptake. Pioglitazone administered daily by oral gavage (20 mg/kg per day) for 3 weeks attenuated the development of hypertension in both the Dahl salt-sensitive (DS) rat (an insulin-resistant model of hypertension) and the one-kidney, one clip rat (a model of hypertension not associated with insulin resistance). Based on euglycemic insulin clamp studies in conscious animals, the glucose clearance rate was increased (P<.05) in pioglitazone-treated DS rats (36±3 mg/kg per minute) compared with control DS rats (27±1 mg/kg per minute). However, pioglitazone did not affect the glucose clearance rate in one-kidney, one clip hypertensive rats. Metformin, an unrelated agent that also improves glucose tolerance, had no significant effect on blood pressure or glucose clearance rate in either DS or one-kidney, one clip rats. Thus, the hypotensive effect of pioglitazone is invariably associated with its capacity to improve insulin-induced glucose utilization. (Hypertension. 1994;24:106-110.)

Key Words • insulin resistance • rats, inbred strains • hypotension, Goldblatt

In humans, both epidemiologic and clinical evidence document an association between hypertension and resistance to insulin-stimulated glucose uptake.1 Similarly, in the rat elevated arterial pressure is associated with insulin resistance. The Dahl salt-sensitive (DS) rat is insulin resistant, whereas both the one-kidney, one clip (1K1C) hypertensive Sprague-Dawley rat and the two-kidney, one clip hypertensive Sprague-Dawley rat are not.2-4 Several5-6 but not all7 investigators have reported that the spontaneously hypertensive rat (SHR) is insulin resistant. Although a number of putative mechanisms have been proposed, it is unclear whether insulin resistance, hyperinsulinemia, or both actually cause hypertension.1 To address this question, we and others have recently evaluated the effects of oral hypoglycemic agents on arterial pressure in several rat models of hypertension. Pioglitazone is a thiazolidinedione derivative that increases insulin sensitivity without stimulating endogenous insulin secretion.8-10 This agent attenuates the development of hypertension in the DS rat11 and also prevents increases in blood pressure in the rat caused by feeding high-carbohydrate or high-fat diets.12,13 Ciglitazone and CS-045, other thiazolidinedione derivatives, also lower blood pressure in the insulin-resistant, obese Zucker rat.14,15 CS-045 also prevents insulin resistance and hypertension in Sprague-Dawley rats fed high-fructose diets.16 Metformin, a chemically unrelated oral hypoglycemic agent that also does not stimulate insulin secretion, reportedly attenuates the development of hypertension in the SHR.17 Conversely, glyburide, a sulfonylurea antidiabetic agent, increases both plasma insulin concentrations and blood pressure in female (but not in male) stroke-prone SHR.18

The purpose of the present study was to define the relation between changes in insulin sensitivity and attenuation of hypertension. We have extended our studies in the DS rat to include the 1K1C Sprague-Dawley rat. In each model, we evaluated the effects of pioglitazone and metformin on both arterial pressure and insulin sensitivity, as assessed by the euglycemic insulin clamp technique.

Methods

Effects of Pioglitazone and Metformin on the Development of Hypertension and on Insulin Sensitivity in the DS Rat

We have previously reported that pioglitazone attenuates the development of hypertension in the DS rat.11 Using an identical protocol in the present study we evaluated the effect of metformin on blood pressure in this animal model.

Male DS rats (Brookhaven strain) were purchased from Harlan Sprague Dawley (Indianapolis, Ind) and arrived shortly after weaning. Initially, all animals were fed 0.45% NaCl (diet No. 88311, Teklad) for 1 week and subsequently a 3% NaCl diet (diet No. 89281, Teklad). The rats were housed in individual cages in a temperature-controlled (22°C) and light-controlled (12 hours on, 12 hours off) small-animal facility. All animals ate and drank (tap water) ad libitum. In half the animals, metformin was placed in the drinking water, and animals received a total daily dose of 300 to 350 mg/kg. To achieve this daily dose, based on the water intake of individual rats, the concentration of metformin in the water varied from 0.3% to 0.4%. Body weights and tail systolic blood pressures were measured weekly for 5 weeks. We have previously...
described our technique for measuring systolic blood pressure by tail plethysmography. At the end of 5 weeks, animals were catheterized for measurement of direct mean arterial pressure in the conscious, unrestrained state. The femoral artery was catheterized (Tygon microbore catheter, 0.015-inch inner diameter and 0.030-inch outer diameter) with rats under methohexital sodium anesthesia (50 mg/kg IP), and the catheter was tunneled subcutaneously to exit at the base of the skull. Animals continued to receive metformin as above, and 4 to 5 days after preparatory surgery, direct arterial pressure was recorded using a model 7 polygraph (Grass Instruments) and a Statham pressure transducer (Gould Inc).

Insulin sensitivity was measured with the euglycemic insulin clamp technique (described below) in additional groups of control, pioglitazone-treated, and metformin-treated DS rats. Because the half-life of metformin is approximately 3 hours,20 measurements of direct arterial pressure and insulin sensitivity were carried out in another group of metformin-treated rats that received an additional dose of metformin (300 mg/kg by oral gavage) 2 hours before the euglycemic clamp study was begun. For the insulin clamp studies, all animals were maintained and described above, and as we have previously described, pioglitazone was dissolved in water (5 mg/mL) and administered for 3 weeks by daily gavage (20 mg/kg).

Effects of Pioglitazone and Metformin on the Development of Hypertension and on Insulin Sensitivity in 1K1C Rats

Sprague-Dawley rats were purchased from Harlan Sprague Dawley. All animals were fed 0.45% NaCl (Teklad diet No. 88311) and were housed in individual cages as above. At age 4 to 5 weeks, the rats were unilaterally nephrectomized by laparotomy under methohexital sodium anesthesia (50 mg/kg IP), and the contralateral renal artery (left) was partially constricted with a 0.44-mm inner diameter silver clip. Beginning 1 week after renal artery clipping, rats were assigned to either a control, pioglitazone, or metformin group. As above, pioglitazone was administered for 3 weeks by daily gavage (20 mg/kg per day), and metformin was administered in the drinking water (300 to 350 mg/kg per day). At the end of 3 weeks, animals were catheterized for measurement of direct arterial pressure and determination of insulin sensitivity. Animals continued to receive pioglitazone or metformin, and direct arterial pressure and subsequently glucose clearance were measured in conscious animals 4 to 5 days after preparatory surgery.

Estimation of Insulin Sensitivity With the Euglycemic Insulin Clamp Technique

In addition to the arterial catheter, a jugular venous catheter (Tygon microbore catheter, 0.015-inch inner diameter and 0.030-inch outer diameter) was also placed 4 to 5 days before study. After a baseline blood sample for measurement of glucose and insulin was obtained, human insulin (Novolin, Novo Nordisk) was infused through the jugular venous catheter for 120 minutes at a rate of 4 μU/min per kilogram. This infusion rate suppresses hepatic glucose production and is the amount of insulin that produces the half-maximal effect to stimulate peripheral glucose utilization.21 During the insulin infusion, 60-μL samples of femoral arterial blood were drawn at 10- to 15-minute intervals for the immediate determination of plasma glucose. Based on the results of these measurements, 10% dextrose was infused at a rate sufficient to maintain plasma glucose concentrations at preinfusion levels. Additional blood (0.8 mL) was obtained after 90 and 120 minutes of the insulin infusion, and plasma was frozen for subsequent measurement of plasma insulin concentrations. Glucose concentrations were measured with a glucose oxidase method (model 23A glucose analyzer, Yellow Springs Instruments). Plasma insulin was measured by radioimmunooassay with a radioimmunoassay kit (INCASTAR Corp). For measurement of fasting insulin concentrations, a rat standard was used, and a porcine standard was used for measurement of plasma insulin concentrations during infusion of human insulin.

Steady-state plasma glucose and insulin values and glucose infusion rates were calculated using data obtained from the final 30 minutes of the insulin infusion. Plasma insulin was measured at 90 and 120 minutes, and plasma glucose was measured at 90, 105, and 120 minutes. Values for both glucose and insulin were averaged for each animal to yield a single value for that animal. Glucose infusion rates during the insulin infusion intervals of 90 to 105 and 105 to 120 minutes were also averaged for each animal.

ANOVA was used to determine statistical significance among more than two groups, and when an overall difference was observed, the significance of specific group differences was determined with Fisher’s probability least significant difference test. Group differences were considered statistically significant at a value of P<.05. The significance of two group comparisons was determined with a t test. Results are presented as mean±SEM.

Results

DS Rats

Over the 5 weeks of study, weight gain of metformin-treated and control rats did not differ (n=12 per group). In contrast to our previously reported results with pioglitazone, in the present study tail systolic blood pressures did not differ significantly in control and metformin-treated DS rats (Fig 1). Direct mean arterial pressures also did not differ significantly in control (130±4 mm Hg) and metformin-treated (123±2 mm Hg) rats (n=9 per group). Similarly, in the protocol in which rats received an additional dose of metformin by gavage 2 hours before the blood pressure measurement was obtained, direct mean arterial pressures again did not differ in control (124±1 mm Hg) and metformin-treated (127±2 mm Hg) rats (n=10 per group). In DS rats before the glucose and insulin infusions, fasting blood glucose and plasma insulin concentrations were lower (P<.05) in pioglitazone-treated than in control animals (Table 1). Fasting blood glucose and insulin concentrations did not differ in metformin-treated and control rats. During the euglycemic insulin clamp infusion, plasma insulin concentrations did not differ significantly among the three groups of DS rats, and in each group blood glucose concentrations did not differ from fasting levels. The computed glucose clearance rate was significantly greater (P<.05) in pioglitaza-
zone-treated DS rats than in metformin-treated or control rats; glucose clearance in metformin-treated and control rats did not differ.

Similarly, in the protocol in which animals received the additional dose of metformin 2 hours before study, comparison of control and metformin-treated rats showed no differences in plasma glucose and insulin concentrations either before or during the insulin infusion. Glucose clearance rates also did not differ in control (34±2 mg/kg per minute) and metformin-treated (33±2 mg/kg per minute) rats.

**1K1C Rats**

Mean body weights and weight gain did not differ in control (n=13), pioglitazone-treated (n=9), and metformin-treated (n=10) animals. Within 1 week after pioglitazone was begun, systolic blood pressures were lower (P<.05) in pioglitazone-treated rats than in the other two groups (Fig 2). This blood pressure reduction by pioglitazone was maintained for 3 weeks, and after this time direct mean arterial pressures were also lower (P<.05) in pioglitazone-treated rats than in control and metformin-treated rats (Table 2). Comparison of control and metformin-treated 1K1C rats showed no differences in systolic or mean arterial blood pressures.

Fasting blood glucose and insulin concentrations in pioglitazone-treated and metformin-treated 1K1C rats did not differ from those in controls (Table 2). During the euglycemic insulin clamp infusion, there were no significant differences of blood glucose or plasma insulin concentrations among the three animal groups. Glucose clearance rates also did not differ significantly among control, pioglitazone-treated, and metformin-treated 1K1C rats.

### Discussion

Chemically diverse compounds that increase insulin sensitivity attenuate the development of hypertension in several rat models. However, it is unclear whether attenuation of hypertension is specifically related to the capacity of these agents to increase insulin-induced glucose uptake or to some other mechanism. We undertook the present study to define the relation between drug-induced changes of insulin sensitivity and the development of hypertension in two rat models: an insulin-resistant model (the DS rat) and an insulin-sensitive model (the 1K1C rat).

The results indicate that there is not an invariable association between attenuation of hypertension and increases of insulin sensitivity, as assessed by the euglycemic insulin clamp technique. In contrast to our earlier observations with pioglitazone, we now report that metformin fails to attenuate the development of hypertension in the DS rat. Also, in contrast to pioglitazone, metformin did not alter peripheral glucose utilization. Similarly, in the 1K1C Sprague-Dawley rat, the development of hypertension was attenuated by pioglitazone but not by metformin although in this animal model neither pioglitazone nor metformin had an effect on insulin sensitivity. Taken together, these results suggest that reduction of arterial pressure by pioglitazone is not directly related to its capacity to increase insulin-induced glucose utilization. However, we cannot exclude the possibility that the hypotensive action of pioglitazone in DS rats might be partly due to increased insulin sensitivity, whereas in the 1K1C model attenuation of hypertension might be due to some other mechanisms.

Pioglitazone and other thiazolidine derivatives have been reported to improve insulin sensitivity in obese Zucker rats, Wistar fatty rats, KKA mice, ob/ob mice, db/db mice, and normal rats. In the present study, based on measurements of fasting blood glucose and plasma insulin concentrations and on assessment of whole-animal glucose clearance with the euglycemic insulin clamp technique in conscious animals, pioglitazone increased insulin sensitivity in the DS rat but not in the 1K1C Sprague-Dawley rat. Conceivably, the different effects of pioglitazone on insulin-stimulated glucose uptake in these two animal models may be related to the underlying differences of insulin sensitivity.

The mechanism by which metformin and other biguanides improve glucose tolerance is not completely understood. The antihyperglycemic action is multifactorial and has been attributed to diminished intestinal absorption of carbohydrates, reduced gluconeogenesis,

### Table 1. Mean Plasma Glucose and Insulin Concentrations and Glucose Clearance Rates During Insulin Clamp Study in Control, Pioglitazone-Treated, and Metformin-Treated DS Rats

<table>
<thead>
<tr>
<th></th>
<th>Plasma Glucose, mmol/L</th>
<th>Plasma Insulin, μU/mL</th>
<th>GCR, (mg/kg)/min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fasting</td>
<td>Clamped</td>
<td>Fasting</td>
</tr>
<tr>
<td>Control (n=11)</td>
<td>8.1±0.1</td>
<td>8.1±0.2</td>
<td>18±4</td>
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<tr>
<td>Pioglitazone (n=11)</td>
<td>7.6±0.1*</td>
<td>7.6±0.1*</td>
<td>5±2†</td>
</tr>
<tr>
<td>Metformin (n=12)</td>
<td>7.8±0.1</td>
<td>7.9±0.1</td>
<td>15±3</td>
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</tbody>
</table>

DS indicates Dahl salt-sensitive; GCR, glucose clearance rate.

*P<.05 vs control.
†P<.05 vs metformin.

**Fig 2.** Line graph shows systolic blood pressures (SBP) in control, pioglitazone-treated, and metformin-treated one-kidney, one clip hypertensive rats. *P<.05 vs control; †P<.05 vs metformin.
TABLE 2. Mean Arterial Pressure, Plasma Glucose and Insulin Concentrations, and Glucose Clearance Rates During Insulin Clamp Study in Control, Pioglitazone-Treated, and Metformin-Treated 1K1C Rats

<table>
<thead>
<tr>
<th></th>
<th>Control (n=9)</th>
<th>Pioglitazone (n=8)</th>
<th>Metformin (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mm Hg</td>
<td>171±6</td>
<td>149±8*</td>
<td>172±6</td>
</tr>
<tr>
<td>Fasting</td>
<td>8.8±0.2</td>
<td>8.4±0.2</td>
<td>9.3±0.2</td>
</tr>
<tr>
<td>Clamped</td>
<td>21±4</td>
<td>27±5</td>
<td>29±6</td>
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<tr>
<td>Plasma Glucose, mmol/L</td>
<td>8.8±0.2</td>
<td>8.4±0.3</td>
<td>9.3±0.2</td>
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<tr>
<td>Fasting</td>
<td>75±8</td>
<td>67±8</td>
<td>85±10</td>
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<tr>
<td>Clamped</td>
<td>35±2</td>
<td>35±2</td>
<td>38±2</td>
</tr>
<tr>
<td>Plasma Insulin, µU/mL</td>
<td>29±6</td>
<td>29±6</td>
<td>29±6</td>
</tr>
<tr>
<td>GCR, (mg/kg)/min</td>
<td>35±2</td>
<td>35±2</td>
<td>35±2</td>
</tr>
</tbody>
</table>

1K1C indicates one-kidney, one clip; MAP, mean arterial pressure; and GCR, glucose clearance rate.

*P<.05 vs control and metformin.

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