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 not 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

Received January 25, 1994; accepted in revised form April 13, 1994.

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In DS rats before the glucose and insulin infusions, tail systolic blood pressure (SBP) was measured and in each group blood glucose concentrations did not differ significantly among the three groups of DS rats. Overall, tail SBP was significantly lower (P<.05) in metformin-treated DS rats (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 pioglia-
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,
and in diabetic animals and humans to increased peripheral glucose uptake.22–24 At the cellular level, metformin has been reported to potentiate insulin action.25 However, in the present study at the whole-animal level, metformin had no demonstrable effect on insulin sensitivity in either the DS rat or the 1K1C hypertensive rat. Peripheral glucose uptake is less sensitive to the action of insulin than the ability of insulin to increase skeletal muscle glycogenolysis and to inhibit hepatic glucose production.26 Our results are consistent with the hypothesis that improvement of glucose tolerance by metformin is related to its effect on glycogenolysis and on hepatic glucose production rather than to peripheral glucose utilization.

Results of the present study do not define the mechanism by which pioglitazone prevents hypertension. We have previously reported that pioglitazone decreases peripheral resistance in the DS rat and suggested that this may be related to the effect of the drug on the growth of vascular smooth muscle cells. Pioglitazone inhibits mitogen-stimulated growth of renal afferent arteriolar smooth muscle cells. Lovastatin, an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase, also attenuates the development of hypertension in the DS rat28 and inhibits proliferation of rat mesangial cells and rat aortic smooth muscle cells.29 Ciglitazone (another thiazolidinedione) abolishes sustained elevations of intracellular calcium induced by platelet-derived growth factor in A172 human glioblastoma cells.14 Conceivably, the capacities of thiazolidinediones to inhibit mitogen-stimulated growth of vascular smooth muscle and to decrease peripheral vascular resistance might be mediated by alterations of intracellular calcium.

The capacity to excrete sodium is diminished in the DS rat,30 and this may be related to a reduction in the cytochrome P-450-dependent ω-hydroxylation of arachidonic acid in the renal medulla.31 Clofibrate, a lipid-lowering agent that also increases insulin sensitivity, has recently been shown to induce the ω-hydroxylation of fatty acids by cytochrome P-450 in the kidney and to prevent the development of hypertension in the DS rat.32 An effect of clofibrate, and other agents that increase insulin sensitivity, on sodium excretion will require further evaluation.

In conclusion, although pioglitazone attenuates the development of hypertension in the DS rat and 1K1C rat, the capacity of this agent to reduce arterial pressure may not be directly related to its capacity to increase whole-body insulin-stimulated glucose uptake. Whatever the mechanism, the potential of a single agent to decrease blood pressure and increase insulin sensitivity has important clinical implications.

### 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>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=9)</td>
<td>171±6</td>
<td>8.8±0.2</td>
<td>21±4</td>
</tr>
<tr>
<td>Pioglitazone (n=8)</td>
<td>149±8*</td>
<td>6.4±0.2</td>
<td>27±5</td>
</tr>
<tr>
<td>Metformin (n=9)</td>
<td>172±6</td>
<td>9.3±0.2</td>
<td>29±6</td>
</tr>
</tbody>
</table>

*P<.05 vs control and metformin.

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

### References

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*Hypertension*. 1994;24:106-110
doi: 10.1161/01.HYP.24.1.106

*Hypertension* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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