Glucose Tolerance and Insulin Action in Rats With Renovascular Hypertension

Thomas A. Buchanan, George F. Sipos, Sam Gadalah, Kay-Pong Yip, Donald J. Marsh, Willa Hsueh, and Richard N. Bergman

To test whether hypertension can cause hyperinsulinemia or insulin resistance, we performed intravenous glucose tolerance tests at 1 month and euglycemic clamps at 3 months after induction of two-kidney, one clip renovascular hypertension in rats. At 1 month, systolic pressure was higher in 21 clipped than in 12 control animals (161±5 mm Hg, range 134–187 mm Hg versus 119±3 mm Hg, range 108–146 mm Hg; p<0.001). Glucose tolerance, assessed as the glucose fractional disappearance rate between 3 and 11 minutes after the glucose injection, was similar in the clipped and sham groups (0.059±0.002 versus 0.056±0.002 min⁻¹, respectively; p>0.4). The total area under the insulin curve during glucose tolerance tests was also similar in the clipped and sham groups (926±95 versus 869±126 microunits/ml×min; p>0.4). There was no significant relation between systolic blood pressure and insulin area during glucose tolerance tests in the clipped group, but there was a positive rectilinear relation in the control group (r=0.66; p=0.01). Fourteen animals had euglycemic clamps 2 months after glucose tolerance tests. At that time, systolic pressure (direct femoral measurement) was higher in the seven clipped animals (189±13 mm Hg versus 122±5 mm Hg in controls; p<0.001). Insulin infusions of 1 and 4 milliunits/min/kg body wt effected similar plasma insulin levels in the two groups. Glucose requirements during 1 milliunit/min/kg insulin were higher in the clipped than the control group (6.1±0.7 versus 2.4±0.6 mg/min/kg, respectively; p<0.001). Glucose requirements during 4 milliunits/min/kg insulin were similar in the two groups (28.4±1.1 versus 26.8±2.8 mg/min/kg; p>0.5). Our data indicate that neither mechanisms leading to renovascular hypertension nor elevated blood pressure per se caused sustained hyperinsulinemia or insulin resistance in this rat model. (Hypertension 1991;18:341-347)

Hypertension is associated with insulin resistance and hyperinsulinemia in lean and obese people.1–11 Whether these abnormalities of carbohydrate metabolism and blood pressure are causally related is currently unknown. Based on effects of acute hyperinsulinemia to reduce renal sodium excretion12–14 and increase circulating catecholamine levels,15–17 it has been proposed that hyperinsulinemia may contribute to the genesis or maintenance of hypertension in some patients. That proposal is supported by studies of weight loss18 and physical training,19 since blood pressure tends to fall in association with reduced insulin levels in both settings. Furthermore, suppression of endogenous hyperinsulinemia with somatostatin has been reported to reduce elevated blood pressures in obese men20 and in rats made hypertensive and insulin resistant by diets high in fructose.21 Thus, it appears that abnormalities of carbohydrate metabolism resulting in hyperinsulinemia may contribute to elevated blood pressure in some forms of hypertension.

On the other hand, there are theoretical reasons to suspect that hypertension might lead to insulin resistance and, in the presence of normal pancreatic B-cell reserve, hyperinsulinemia. These reasons follow from a consideration of the normal steps involved in insulin-mediated glucose uptake. At the tissue level, insulin appears to augment blood flow to skeletal muscle,22 a tissue that predominates in glucose uptake during physiological hyperinsulinemia.23 The effect on blood flow may account for up to 40% of insulin-stimulated glucose uptake in normal subjects.22 Hypertension, particularly forms that are characterized by high vascular resistance, could lead to a reduction of insulin's effect on blood flow and thus to reduced glucose uptake.
Animals before the first pressure recording was made. It is known about this transport process, capillary damage induced by long-standing hypertension could disrupt normal insulin delivery to target tissues, thereby reducing insulin's effect on glucose uptake. At the cellular level, insulin's effect on glucose transport may be adversely affected by alterations of intracellular calcium concentrations, which may be abnormal in some forms of hypertension. Thus, hypertension could lead to a deterioration of insulin's action on glucose uptake, leading to compensatory hyperinsulinemia and, perhaps, worsening of the hypertension. The present study was carried out to test whether one form of experimental hypertension, renovascular hypertension, can lead to hyperinsulinemia or insulin resistance.

Methods

Animals
Normal male Sprague-Dawley rats (Harlan Sprague Dawley, Inc., Indianapolis, Ind.) were housed singly in a light- (12-hour light/dark cycle, 6:00 AM to 6:00 PM) and temperature-controlled (22±2°C) environment. They had free access to food (Teklad Premier, Wayne Laboratory Foods, Indianapolis, Ind.) and tap water except during intravenous glucose tolerance tests (IVGTTs) and glucose clamps. All procedures were approved by the Animal Care Committee of the University of Southern California.

Induction of Hypertension
Twenty-one rats weighing 150–175 g underwent placement of a 0.2 mm metal clip around the left renal artery during general anesthesia with ketamine and xylazine. Clips were placed approximately 5–7 mm from the renal hilum and care was taken to avoid damage to the renal or hepatic parenchyma during the procedure. Twelve animals of similar weight underwent sham surgery that involved identical anesthesia and isolation of the left renal artery without clipping. Surgeries were performed by an investigator who coded the experimental group of each animal so that blood pressure measurements and metabolic assessments could be performed in a blinded fashion. Location of renal artery clips was confirmed at autopsy in all animals.

Blood Pressure Measurements
For animals undergoing IVGTTs, systolic blood pressure was measured in the morning by a tail-cuff device that used a pneumatic sensor to detect the tail arterial pulse and an aneroid sphygmomanometer for measurement of the blood pressure (Bel-Art, Pequannock, N.J.). Animals were placed in a Plexiglas restraining cage at an ambient air temperature of 30–31°C for at least 20 minutes before each blood pressure determination. Animals were adapted to the blood pressure measurement procedure three times before the first pressure recording was made.

In animals studied with glucose clamps, blood pressure was measured by a direct arterial technique on the day after the glucose clamp. Briefly, animals were anesthetized with pentobarbital, and a polyethylene catheter (PE-50, Clay-Adams Co., Parsippany, N.J.) filled with heparin-saline was placed in a femoral artery. Animals were allowed 4 hours to recover from the procedure, then blood pressure was recorded directly from the arterial catheter, which was connected to a pressure transducer, strain-gauge amplifier, and chart recorder (Grass Instruments, Quincy, Mass.).

Preparation for Metabolic Studies
At least 84 hours before IVGTTs and glucose clamps, animals were placed in special cages with the distal third of each animal's tail drawn through a hole in the cage bottom and secured there with a rubber stopper. This arrangement was required for access to tail blood vessels during IVGTTs and clamps; it allowed the animals freedom to move about and did not restrict access to food or water. Preliminary studies in normal male rats revealed that placement in tail restraints caused a slight reduction in food intake and weight gain during the first 24 hours, followed by a return of both parameters to prerestraint levels thereafter.

Four hours before IVGTTs and clamps, animals underwent placement of a tail artery blood sampling catheter and one (for IVGTTs) or two (for glucose clamps) tail vein infusion catheters (PE-10, Clay-Adams). Catheters were placed percutaneously during local anesthesia with lidocaine while animals were briefly restrained in a towel. After catheter placement, animals were returned to their cages and were free to move about during the IVGTTs and glucose clamps.

Intravenous Glucose Tolerance Tests
IVGTTs were performed to assess pancreatic B-cell responses to glucose and glucose tolerance 1 month after renovascular clipping or sham surgery. Rats were in a fasting condition for 5 hours before receiving a bolus injection of dextrose (300 mg/kg body wt as a 10% solution in water) into one tail vein over 30 seconds. An injection of tolbutamide (3.5 mg/kg) was given 12 minutes after the glucose injection to assess the insulin response to a non-glucose secretagogue. Tail arterial blood samples (300 μl) were obtained at −1, 1, 3, 5, 8, 11, 13, 15, 17, 24, 40, and 60 minutes relative to the glucose injection. Plasma from these samples was assayed immediately for glucose, then frozen at −20°C for subsequent insulin measurement. Tail catheters were removed immediately after IVGTTs, and animals to be studied with glucose clamps were housed in normal cages without tail restraints until 84 hours before glucose clamps, when they were returned to special cages with tail restraints.

Glucose Clamps
Euglycemic, hyperinsulinemic clamps were performed to assess whole-body insulin sensitivity.
months after renovascular surgery. Rats were in a fasting condition for 5 hours before receiving two sequential 120-minute infusions of crystalline human insulin (Novolin, Novo Nordisk, Princeton, N.J.) through one tail vein. The initial infusion was administered at a rate of 1 milliunit/min/kg body wt. The second infusion was given at 4 milliunits · min⁻¹ · kg⁻¹. During the insulin infusions, 40-µl samples of tail arterial blood were drawn at 10-minute intervals for the immediate determination of plasma glucose. Based on those determinations, dextrose (10% wt/vol in water) was infused at a rate sufficient to maintain plasma glucose at the preinfusion level. Additional blood (400 µl) was obtained after 100, 110, and 120 minutes of each insulin infusion. Plasma from those samples was frozen for insulin measurement.

**Analytical Techniques**

Glucose was measured by glucose oxidase (Beckman Glucose Analyzer II, Beckman Instruments, Fullerton, Calif.). Insulin was measured by a charcoal precipitation radioimmunoassay technique. The insulin concentrations in samples obtained during human insulin infusions were calculated using a human insulin standard; all other concentrations were calculated against a rat insulin standard (both from Novo Nordisk, Wilton, Conn.).

**Data Analysis**

Glucose tolerance was calculated as the slope (×100 = $K_g$) of the line relating the natural log of the plasma glucose concentration to time between 3 and 11 minutes after the glucose injection. The insulin response to intravenous glucose and tolbutamide was calculated as the area under the plasma insulin curve from −1 to 60 minutes relative to the glucose injection.

Steady-state plasma glucose and insulin values and glucose infusion rates during glucose clamps were calculated using data obtained from the final 30 minutes of each 2-hour insulin infusion. For plasma glucose and insulin, values from the 100-, 110-, and 120-minute plasma samples for an individual animal were averaged to give a single value for that animal. The individual averages were used to calculate group means for steady-state glucose and insulin. Similar calculations were made for the glucose infusion rates during the 90–100-, 100–110-, and 110–120-minute intervals of each insulin infusion.

Body weight, blood pressure, 5-hour fasted plasma glucose and insulin concentrations, $K_g$, IVGTT insulin area, steady-state plasma glucose and insulin, and glucose infusion rates during glucose clamps were compared between clipped and sham groups by a nonparametric method (Mann-Whitney $U$ test). Data are presented as mean±SEM.

**Results**

**One-Month Studies**

Means of body weight were similar between the 21 renal artery clipped and the 12 sham-operated rats 1 month after surgery (Table 1). The mean of systolic blood pressures was higher in the clipped group (Table 1) (range 134–187 mm Hg) than in the sham-operated animals (range 108–146 mm Hg).

Five-hour fasting plasma glucose and insulin levels at the start of the IVGTTs were virtually identical in the sham and clipped groups (Table 1). Plasma glucose and insulin patterns after the glucose and tolbutamide injections were likewise very similar in the two groups (Figure 1). Thus, the $K_g$ values between 3 and 11 minutes (5.58±0.24 versus 5.93±0.18 min⁻¹) and the total areas under the insulin curves during the IVGTTs (869±126 versus 926±95 microunits/ml·min) did not differ significantly between sham and clipped groups, respectively ($p>0.4$ in each case). Since there was a wide range of blood pressures in the clipped group, we examined the relations between blood pressure and IVGTT insulin area to see whether animals with the highest blood pressures also had the greatest insulin responses to glucose. We found no evidence for such a relation in the clipped animals, despite systolic blood pressures as high as 187 mm Hg (Figure 2). By contrast, there was a significant positive relation between blood pressure and insulin area in the sham

<table>
<thead>
<tr>
<th>Study group</th>
<th>Body weight (g)</th>
<th>Systolic blood pressure (mm Hg)</th>
<th>Fasting glucose (mg/dl)</th>
<th>Fasting insulin (microunits/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Month</td>
<td></td>
<td></td>
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<tr>
<td>Control (n=12)</td>
<td>312±9</td>
<td>121±5</td>
<td>138±3</td>
<td>36±4</td>
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<tr>
<td>Clipped (n=21)</td>
<td>315±5</td>
<td>161±6*</td>
<td>140±2</td>
<td>32±3</td>
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<tr>
<td>3 Months</td>
<td></td>
<td></td>
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<tr>
<td>Control (n=7)</td>
<td>404±12</td>
<td>122±5</td>
<td>139±3</td>
<td>29±3</td>
</tr>
<tr>
<td>Clipped (n=7)</td>
<td>403±16</td>
<td>189±13*</td>
<td>140±2</td>
<td>25±2</td>
</tr>
</tbody>
</table>

One month denotes animals studied with intravenous glucose tolerance tests (IVGTTs) 1 month after sham or renal artery clip surgery. Three months denotes animals studied with euglycemic clamps at 3 months. One-month blood pressures were measured by a tail-cuff occlusion method. Three-month blood pressures were measured directly from the femoral artery of conscious animals, as described in "Methods." Body weights were measured in the morning on the days of IVGTTs and euglycemic clamps.

*p<0.001 vs. control group.
FIGURE 1. Graphs show plasma glucose and insulin during intravenous glucose tolerance tests (IVGTTs) in sham-operated control rats and rats with renovascular hypertension (Clipped) for 1 month. Dextrose (300 mg/kg body wt) was injected intravenously into five-hour-fasted, conscious animals at t=0. Tolbutamide (3.5 mg/kg) was injected 12 minutes after dextrose. Upper panel insert: Fractional glucose disappearance ($K_{g}$) between 3 and 11 minutes after glucose injection. Lower panel insert: Integrated area under insulin curve during IVGTTs. No significant difference was noted between groups for any of the parameters displayed.

FIGURE 2. Scatterplot shows relation between tail-cuff systolic blood pressure (BP) and intravenous glucose tolerance test (IVGTT) insulin area in sham and clip groups at 1 month. Solid line depicts best rectilinear fit for sham group ($p=0.01$); dashed line depicts best fit for clipped rats (NS).

group ($r=0.66, p=0.01$; Figure 2). No significant relation was detected between $K_{g}$ and blood pressure in the control ($r=0.08$) or the clipped ($r=0.3$) group. Thus, 1 month of two-kidney, one clip hypertension had no detectable effect on intravenous glucose tolerance but appeared to disrupt the relation between blood pressure and insulin responses that was present in normal animals.

Three-Month Studies

Seven control and seven clipped animals were studied with euglycemic clamps after 3 months of hypertension in the clipped group. At that time, the means of systolic blood pressures (direct femoral measurements) were 122±5 mm Hg in the controls and 189±13 mm Hg in the clipped group (Table 1). Means of body weights (Table 1) and 5-hour fasting plasma glucose and insulin (Figure 3) were similar in the control and clipped groups. Insulin infusions at a rate of 1 milliunit/min/kg resulted in insulin concentrations of 31±4 and 31±2 microunits/ml in control and clipped animals, respectively (Figure 3). The mean of glucose infusion rates required to maintain euglycemia in the face of these circulating insulin levels was significantly less in the controls than in the clipped rats (2.4±0.6 versus 6.1±0.7 mg/min/kg; $p<0.001$; Figure 3). High-dose insulin infusions resulted in plasma insulin concentrations of 80±3 and 81±4 microunits/ml in control and clipped groups, respectively (Figure 3). Glucose infusion rates needed to maintain euglycemia in the face of these higher insulin levels did not differ significantly between groups (26.8±2.8 versus 28.4±1.1 mg/min/kg; $p>0.5$; Figure 3). During the high-dose insulin infusions in control animals, there was an inverse relation of borderline statistical significance ($r=-0.59; p=0.09$) between the glucose infusion/plasma insulin ratio (a measure of insulin sensitivity) and systolic blood pressure. No significant relation was noted in the clipped group ($r=0.36; p>0.3$). Thus, although insulin action during the higher dose insulin infusions tended to be inversely related to blood pressure in controls, no such relation existed in hypertensive animals 3 months after clipping, and we found no evidence for resistance to insulin's effect on glucose metabolism in this rat model of induced hypertension.

Discussion

The present study was designed to test whether blood pressure elevation can lead to glucose intolerance, hyperinsulinemia, or insulin resistance. We selected the two-kidney, one clip Goldblatt model of hypertension, which is characterized in the acute phase by activation of the renin-angiotensin system, leading to vasoconstriction and hypertension, and in the chronic phase by variable sodium and water retention along with increased vascular resistance.29,30 We measured glucose and insulin responses to intravenous glucose 1 month after renal artery clipping and observed virtually identical insu-
FiguRe 3. Bar graphs show plasma glucose and insulin concentrations and glucose infusion rates during euglycemic clamps in control and renal artery-clipped rats at 3 months. Low Dose and High Dose, values obtained during the final 30 minutes of sequential 120-minute insulin infusions at 1 and 4 milliunits/min/kg, respectively. *p<0.001 vs. control group.

lin and glucose patterns in hypertensive and control animals. These findings are indicative of similar pancreatic B-cell function, as well as similar effects of insulin to mediate glucose disappearance in the two groups.

Reasoning that perhaps only the most hypertensive animals would manifest abnormal insulin responses, we looked for a relation between blood pressure and the integrated insulin response to injected glucose and found none in the clipped animals. By contrast, we found a significant direct relation between blood pressure and plasma insulin area in the control animals. This relation is similar to those reported in some normotensive human populations. Reasoning that perhaps only the most hypertensive animals would manifest abnormal insulin responses, we looked for a relation between blood pressure and the integrated insulin response to injected glucose and found none in the clipped animals. By contrast, we found a significant direct relation between blood pressure and plasma insulin area in the control animals. This relation is similar to those reported in some normotensive human populations. The mechanism responsible for the association between blood pressure and insulin in normal animals is not apparent from the present study but could reflect the effects of insulin on renal sodium handling or sympathetic nervous system activity. The lack of a significant insulin–blood pressure relation in clipped animals indicates that hypertension was maintained by mechanisms that neither involved nor impacted on the insulin-glucose axis. This finding suggests that neither blood pressure elevation per se nor mechanisms similar to those involved in the development of short-term renovascular hypertension (e.g., activation of the renin-angiotensin system) can be invoked to explain the insulin–blood pressure relation in normal animals. However, we cannot exclude the possibility that a brief period of insulin resistance or hyperinsulinemia occurred before our initial testing at 1 month.

Since abnormalities of vascular structure and function that could lead to impaired insulin action have been reported in rats with chronic renovascular hypertension, we measured insulin’s effect on glucose metabolism directly with the euglycemic clamp technique 3 months after renal artery clipping. Based on the data of Smith et al., we used two insulin infusion rates to effect plasma insulin concentrations that approximated the half-maximal effect of insulin to suppress hepatic glucose production (30–40 microunits/ml; low-dose insulin) and the half-maximal effect to stimulate peripheral glucose use (70–80 microunits/ml; high-dose insulin). The greater glucose requirements of clipped animals during the low-dose insulin suggests a greater effect of insulin to suppress endogenous glucose production in that group, although studies with labeled glucose will be required to verify that suggestion. Similar glucose requirements during the higher dose insulin infusions, which should have completely suppressed hepatic glucose production, indicate similar insulin-mediated glucose disposal in sham and clipped animals. Since we did not study the effect of insulin concentrations above the physiological range, we cannot exclude the possibility that maximal insulin responsiveness may have differed between control and hypertensive rats. However, our findings of greater insulin sensitivity in hypertensive animals during low-dose insulin infusions and similar sensitivities in clipped and control animals during higher dose insulin virtually exclude the possibility that insulin action over the physiological range that we studied was impaired by 3 months of renovascular hypertension.

Our findings are in agreement with limited data available about insulin levels in humans with renovascular hypertension. Marigliano et al. reported that a small number of patients with renovascular hypertension had fasting insulin levels similar to those of normotensive people of similar relative weight and fasting glycemia. On the other hand, patients with essential hypertension had elevated fasting insulin levels, a finding usually associated with insulin resistance, but not invariably found in patients with essential hypertension. No direct measures of insulin sensitivity were reported by Marigliano et al. Our data provide direct measures of insulin sensitivity and nutrient-stimulated insulin responses in renovascular hypertension and indicate no abnormalities of either parameter.
The normal insulin levels and insulin sensitivity of hypertensive animals in this study suggest that the association among hypertension, insulin resistance, and hyperinsulinemia that has been noted in other animal models of hypertension and in humans is not mediated by the effects of elevated blood pressure per se. However, that conclusion must be drawn with some caution for two reasons. First, our studies were relatively short in duration. It is possible that more prolonged or severe hypertension, perhaps in association with other risk factors favoring the development of vascular disease, could result in sufficient vascular damage to impair insulin delivery to target tissues. Second, this renovascular model is clearly not representative of all forms of hypertension. For example, low renin forms of hypertension may be characterized by different circulating calcium levels, intracellular calcium concentrations, and blood pressure responses to sodium than are high renin forms. It is possible that hyperinsulinemia is operative in some forms of hypertension, particularly those characterized by sodium sensitivity of blood pressure. Our data emphasize the need for careful characterization of the types of hypertension that are associated with insulin resistance so that we can better assess possible mechanistic links between abnormal carbohydrate metabolism and elevated blood pressure. Based on the present study, renovascular hypertension does not seem to be important in that regard.

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