Isradipine and insulin sensitivity in hypertensive rats

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1Hypertension Research Unit and 2Diabetes Research Unit, Laval University Medical Research Center, Centre Hospitalier de l'Université, Laval University, Ste-Foy, Quebec, Canada G1V 4G2

Pître, Maryse, Nathalie Gaudreau, Marta Santure, Andre Nadeau, and Helene Bachelard. Isradipine and insulin sensitivity in hypertensive rats. Am. J. Physiol. 276 (Endocrinol. Metab. 39): E1038–E1048, 1999.—The present study was designed to investigate the effect of a reduction in blood pressure, by using the calcium channel antagonist isradipine, on insulin sensitivity and vascular responses to insulin in conscious spontaneously hypertensive male rats (SHR). The rats were instrumented with intravascular catheters and pulsed Doppler flow probes to measure blood pressure, heart rate, and blood flows. Insulin sensitivity was assessed by the euglycemic-hyperinsulinemic clamp technique. Two groups of rats received isradipine at a dose of 0.05 or 0.15 mg·kg⁻¹·h⁻¹, whereas a third group received a continuous infusion of vehicle (15% DMSO). Both doses of isradipine were found to decrease mean blood pressure (−25 ± 4 mmHg at the dose of 0.05 mg·kg⁻¹·h⁻¹ and −20 ± 2 mmHg at the dose of 0.15 mg·kg⁻¹·h⁻¹) and to improve insulin sensitivity. Moreover, in the rats treated with the low dose of isradipine, we observed vasodilations in renal, superior mesenteric, and hindquarter vascular beds. In the untreated group, the euglycemic infusion of insulin (4 mU·kg⁻¹·min⁻¹) was found to cause vasoconstrictions in superior mesenteric and hindquarter vascular beds, but no changes in mean blood pressure, heart rate, or renal vascular conductance were found. In contrast, in the isradipine-treated groups, the same dose of insulin was found to produce vasodilations in the renal vascular bed and to abolish the vasoconstrictor responses previously observed. We concluded that short-term treatment with isradipine in SHR can lower blood pressure and improve insulin sensitivity, mainly through hemodynamic factors, as supported by experiments with hydralazine as a positive vasodilator control.

insulin resistance; hypertension; blood flow; vasodilation

The nature (fortuitous or causal) and significance of this close association between hypertension, insulin resistance, and hyperinsulinemia remain unclear and largely controversial. Among the proposed hypotheses, it has been suggested that this relationship could be secondary to hemodynamic factors (3, 26). Indeed, several studies have reported a positive correlation between blood flow in skeletal muscle and insulin sensitivity (3, 25, 26), as well as between insulin sensitivity and skeletal muscle capillary density (35). Thus, considering that blood flow is an important determinant of glucose uptake into skeletal muscle (3, 25, 26), it has been proposed that microcirculatory changes in skeletal muscle, secondary to vascular hypertrophy and rarefaction, could contribute to insulin resistance by limiting blood flow distribution and, thereby, reduce insulin and glucose delivery to insulin-sensitive tissues (13, 26). Skeletal muscle capillary rarefaction has been found in numerous experimental models of hypertension (20, 39) and in biopsies of skeletal muscle of patients with hypertension (35). Moreover, several laboratories, including ours, have reported that insulin exerts vasodilatory effects (1, 3, 25, 34, 36), which are impaired in insulin-resistant states (3, 30, 31, 36). It is suggested that, through its vasodilator action, insulin could enhance its own ability to promote glucose uptake in skeletal muscle by increasing the insulin and glucose distribution to a greater mass of previously underperfused muscles (3, 31).

Therefore, considering the interrelationship between insulin resistance, skeletal muscle blood flow, and blood pressure, and in continuity with our own recent results showing a reduction in insulin sensitivity as well as an impairment in the insulin-mediated skeletal muscle vasodilation in the SHR (36), it was of interest to examine the effect of a reduction in blood pressure, by using a specific antihypertensive drug, isradipine, on insulin sensitivity and vascular responses in the SHR. Isradipine is a calcium channel antagonist belonging to the dihydropyridine group. It selectively relaxes vascular smooth muscle cells of the resistance vessels (21). Because skeletal muscle blood flow is considered to play a major role in determining insulin-mediated glucose uptake, one would expect that drugs that decrease blood pressure level, by reducing peripheral vascular resistance and increasing blood flow as well as reducing the cytosolic calcium content, may have beneficial effects on insulin resistance (12). However, there are many conflicting reports about the effects of dihydropyridine calcium channel antagonists on insulin sensitivity and glycemic control. Some calcium channel antago-
nists have been shown to impair glucose tolerance and insulin sensitivity (5), whereas others have been reported to have no significant effect on insulin sensitivity (15, 28). There is also evidence that dihydropyridines may enhance glucose tolerance and insulin sensitivity (4, 22).

Therefore, we have undertaken the present study to investigate the short-term effect of isradipine on blood pressure, regional blood flow, insulin sensitivity, and vascular responses to insulin with the euglycemic-hyperinsulinenic clamp in conscious SHR chronically instrumented with intravascular catheters and pulsed Doppler flow probes to continuously record blood pressure, heart rate, and regional blood flows. These in vivo conscious animal procedures involving both metabolic and vascular response determinations simultaneously in the same animal are original and add something new to what has already been shown with isradipine and other calcium antagonists. These experimental approaches constitute a unique and powerful tool to investigate the interactions among insulin sensitivity, blood flow, and the hemodynamic responses to insulin.

METHODS

All surgical and experimental procedures followed institutional animal care guidelines. Male SHR rats (aged 12–14 wk and weighing 250–300 g) were purchased from Charles River Canada. The rats were anesthetized with a mixture of ketamine and xylazine (100 and 10 mg/kg, respectively, ip, supplemented as required) and had pulsed Doppler flow probes implanted to monitor changes in renal, superior mesenteric, and hindquarter blood flows, according to the method previously developed by Gardiner and Bennett (17) and as described in detail previously (36). At least 7 days later, the rats were reanesthetized with a mixture of ketamine and xylazine (100 and 10 mg/kg, respectively, ip, supplemented as required). The leads of the implanted probes were soldered to a microconnector (Microtech), and three separate catheters were implanted in the right jugular vein (for drug infusion) and one catheter was implanted in the distal abdominal aorta via the left femoral artery (for measurement of blood pressure and heart rate). The catheters were tunneled subcutaneously to emerge at the same point as the Doppler probe wires. The microconnector, soldered to the Doppler probe wires, was clamped in a custom-made harness worn by the rat, and the catheters were passed through a flexible, protecting spring attached to the harness. The rats were given intramuscular injections of ampicillin (7 mg/kg) and flunixin (1 mg/kg) and returned to their home cages. Experiments began at =48 h after this last surgical step. Throughout the experiments, nine variables were recorded (heart rate, phasic and mean blood pressures, and phasic and mean Doppler shift signals from renal, superior mesenteric, and hindquarter probes) with a Biopac data acquisition and analysis system (model MP 100, AcqKnowledge software version 3.1). The purpose of recording phasic Doppler shift signals was to ensure that they were of an acceptable quality during the experiments. At selected time points, heart rate, mean blood pressure, and mean Doppler shifts were measured (averaged over 20 s) and related to the preclamp baseline value (absolute changes for the former 2 variables, percentages for the Doppler shifts). In addition, the mean Doppler shift was divided by mean blood pressure to obtain the vascular conductance changes for renal, mesenteric, and hindquarters vascular beds (18). The rats were allowed free access to water but not food for the duration of the experiment.

Experimental Protocols

Euglycemic-hyperinsulinenic clamp studies. The rats were deprived of food for 12–14 h overnight before the glucose clamp study was begun. Six separate groups of SHR were used in this study. Before each experiment, blood glucose and plasma insulin were determined and the resting heart rate, blood pressure, and regional blood flows were recorded over 20 min in the quiet, unrestrained, and unsedated rats. After that time, one group of rats (n = 16) received a continuous intravenous infusion of isradipine in a dose of 0.05 mg·kg\(^{-1}\)·h\(^{-1}\), a second group of rats (n = 19) received isradipine at a dose of 0.15 mg·kg\(^{-1}\)·h\(^{-1}\), and a third group of rats (n = 22) received a continuous intravenous infusion of 15% DMSO (the vehicle used to dissolve isradipine) and served as a control for the drug treatment groups. The rate of infusion was 0.3 ml/h. Each group of rats was divided in two subgroups. The euglycemic-hyperinsulinenic clamp was performed in three subgroups of rats, a first subgroup receiving the vehicle only (n = 10), a second subgroup receiving 0.05 mg·kg\(^{-1}\)·h\(^{-1}\) of isradipine (n = 8), and a third subgroup treated with isradipine at the dose of 0.15 mg·kg\(^{-1}\)·h\(^{-1}\) (n = 10). The other subgroups of vehicle (n = 12) or isradipinetreated rats (n = 8 for isradipine at the dose of 0.05 mg·kg\(^{-1}\)·h\(^{-1}\) and n = 9 for isradipine at the dose of 0.15 mg·kg\(^{-1}\)·h\(^{-1}\) were infused with saline-0.2% BSA, instead of insulin and dextrose, to match approximately the saline load delivered during the clamp studies. These animals were treated in the same way as the subgroups receiving euglycemic infusions of insulin.

Sixty minutes after the beginning of intravenous infusion of isradipine or vehicle, the euglycemic-hyperinsulinenic clamp was then carried out over 2 h in the designated subgroups of rats, while heart rate, blood pressure, and blood flows were measured continuously. Thus, soon after basal measurements of blood glucose and plasma insulin concentrations (measured under drug treatment), each rat received a continuous infusion of regular porcine insulin (Iletin II, 100 mU·ml\(^{-1}\)) and aminoguanine (N) at a rate of 4 µU·kg\(^{-1}\)·min\(^{-1}\). The insulin solution was diluted to the appropriate concentration in saline (0.9% NaCl) containing 0.2% BSA to prevent the adsorption of insulin to the glassware and plastic surfaces. Insulin was infused with a syringe infusion pump (Razel, model A-99) from a reservoir (5-ml syringe) through polyethylene tubing (0.28 mm ID, Clay Adams). The infusion apparatus was calibrated to provide an infusate range of 20 µl/min at the end of the tubing. Ten minutes after the insulin infusion was begun, a 50% dextrose solution (made up with saline) was infused at variable rates to maintain blood glucose at the baseline level (i.e., the preclamp level) according to frequent arterial blood glucose determinations performed at 5-min intervals (with a glucometer Elite, Miles Canada). The glucose infusion rate ranged between 12 and 22 µl/min. In control experiments, the dextrose solution was replaced with saline and infused at a rate of 17 µl/min to approximate the saline load delivered during the clamp studies. The clamp studies were carried out for 120 min to achieve steady-state glucose infusion rates, and the whole body insulin sensitivity of each rat was assessed on the basis of data obtained over the last 60 min of each study. The amount of glucose required to maintain euglycemia during the last hour of the clamp, which corresponded to a steady-state concentration of insulin, was used as an index of insulin sensitivity. Blood samples (0.3 ml)
Table 1. Cardiovascular effects of iv infusion of isradipine or vehicle in conscious, spontaneously hypertensive rats

<table>
<thead>
<tr>
<th></th>
<th>Vehicle (n = 22)</th>
<th>Isradipine, 0.05 mg·kg⁻¹·h⁻¹ (n = 16)</th>
<th>Isradipine, 0.15 mg·kg⁻¹·h⁻¹ (n = 19)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>330 ± 7</td>
<td>340 ± 9</td>
<td>332 ± 10</td>
</tr>
<tr>
<td>Mean blood pressure, mmHg</td>
<td>133 ± 2</td>
<td>133 ± 2</td>
<td>133 ± 3</td>
</tr>
<tr>
<td>Shift, kHz</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal Doppler</td>
<td>7.3 ± 0.8</td>
<td>7.3 ± 0.8</td>
<td>6.0 ± 0.8</td>
</tr>
<tr>
<td>Mesenteric Doppler</td>
<td>12.5 ± 1.3</td>
<td>12.6 ± 1.2</td>
<td>10.7 ± 1.5</td>
</tr>
<tr>
<td>Hindquarter Doppler</td>
<td>7.2 ± 1.0</td>
<td>7.6 ± 1.1</td>
<td>6.1 ± 0.5</td>
</tr>
<tr>
<td>Conductance, 10⁵ KHz/mmHg</td>
<td>55 ± 7</td>
<td>56 ± 6</td>
<td>45 ± 6</td>
</tr>
<tr>
<td>Renal vascular</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mesenteric vascular</td>
<td>94 ± 10</td>
<td>95 ± 9</td>
<td>83 ± 12</td>
</tr>
<tr>
<td>Hindquarter vascular</td>
<td>55 ± 8</td>
<td>58 ± 9</td>
<td>46 ± 4</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of rats. Before, cardiovascular variables measured in resting rats before any drug treatment. After, same cardiovascular variables measured 60 min after the beginning of a continuous iv infusion of vehicle (DMSO 15%) or isradipine. *P < 0.05 vs. before, Student's t-test for paired data.

were collected before intravenous infusion of isradipine or vehicle, just before the beginning of the clamp, and at timed (20 min) intervals during the 120-min euglycemic-hyperinsulinemic clamp for analysis of plasma glucose and insulin concentrations. Red blood cells from these samples were resuspended in saline after centrifugation and immediately returned to the rat.

Effect of hydralazine. To test whether the effects of isradipine were due to hemodynamic factors or were caused by reduction in intracellular calcium concentration, we carried out a new series of experiments with a positive vasodilator control, hydralazine. Two separate groups of SHR were used in this study. One group of rats (n = 17) received a continuous intravenous infusion of hydralazine (0.5 mg/kg iv bolus, 0.25 mg·kg⁻¹·h⁻¹ infusion), and a second group of rats (n = 11) received a continuous intravenous infusion of saline (the vehicle used to dissolve hydralazine) and served as a control for the drug treatment group. The rate of infusion was 0.3 ml/h and was started 60 min before the beginning of the euglycemic hyperinsulinemic clamp. The group of rats treated with hydralazine was divided into two subgroups. The euglycemic hyperinsulinemic clamp was performed in the control group and a subgroup of rats receiving hydralazine (n = 8). The other subgroup of hydralazine-treated rats (n = 9) was infused with saline-0.2% BSA, instead of insulin and dextrose, to match approximately the saline load delivered during the clamp studies. These animals were treated in the same way as the subgroup receiving euglycemic infusion of insulin. The euglycemic hyperinsulinemic clamp was then carried out over 2 h, as previously described.

Analytic methods. Blood for plasma glucose and insulin determinations in the basal state and during insulin infusion was drawn, put in untreated polypropylene tubes, and centrifuged with an Eppendorf microcentrifuge (Minimax, International Equipment). Plasma was stored at −20°C until assay. The glucose concentration of the supernatant was measured by the glucose oxidase method with a glucose analyzer (Technicon RA-XT), and plasma insulin level was measured by radioimmunoassay with porcine insulin standards and polyethylene glycol for separation.

Data analysis. Values are expressed as means ± SE; n is the number of observations. Data describing the biological characteristics of the rats were evaluated with Student’s t-test for paired data, whereas results obtained over time, such as those from cardiovascular responses to insulin in isradipine-, hydralazine-, or vehicle-treated rats, were analyzed for statistical significance by an analysis of variance (ANOVA) for repeated measurements. Post hoc comparisons were made with Fisher’s test. A P value < 0.05 was taken to indicate a significant difference.

RESULTS

Resting values for cardiovascular variables measured before and 60 min after the beginning of a continuous intravenous infusion of vehicle (15% DMSO) or isradipine in the three groups of rats are shown in Table 1. As expected, the infusion of isradipine caused a significant decrease in basal mean blood pressure values in both groups of isradipine-treated rats. These changes were accompanied by significant increases in heart rate and renal, superior mesenteric, and hindquarter vascular conductances. However, these changes were observed with the low dose of isradipine only. The increases in renal, superior mesenteric, and hindquarter vascular conductances observed in the groups receiving the high dose of isradipine did not reach the level of significance. Moreover, the continuous infusion of 15% DMSO had no effect on any measured or calculated cardiovascular variables.

Hemodynamic Responses to Insulin Infusion During the Euglycemic-Hyperinsulinemic Clamp Period

In the group of rats receiving a continuous infusion of vehicle (n = 10) instead of isradipine, we observed that euglycemic infusion of insulin at the dose of 4 mU·kg⁻¹·min⁻¹ had no effect on heart rate, mean blood pressure, or renal blood flow, whereas slight but significant decreases in superior mesenteric (significant at 45 and 75–90 min) and hindquarter (significant at 15 and 60–75 min and 105–120 min) flows were observed when compared with the effects of control infusion of saline-0.2% BSA (Fig. 1A). The maximum decreases in superior mesenteric (−11 ± 3%) and hindquarter (−12 ± 5%) flows were reached 45 and 75 min, respectively, after the start of insulin infusion. Moreover, significant falls in superior mesenteric (significant at 15–45 and 75–90 min) and hindquarter (significant at 15 and 60–120 min) vascular conductances occurred, but no change in renal vascular conductance was seen.

Values in both groups of isradipine-treated rats. These results were made with Fisher’s test. A P value < 0.05 vs. the Euglycemic-Hyperinsulinemic Clamp Period.
The maximum decrease in superior mesenteric vascular conductance (14 ± 3%) was reached 45 min after the start of insulin infusion. The maximum decrease in hindquarter vascular conductance (15 ± 5%) was observed 75 min after beginning insulin infusion.

**Effect of Isradipine**

In the groups of rats receiving isradipine at a dose of 0.05 mg·kg⁻¹·min⁻¹, we found that euglycemic infusion of insulin (4 mU·kg⁻¹·min⁻¹) had no effect on heart rate or mean blood pressure, when compared with the effects of control infusion of saline-0.2% BSA (Fig. 1B). Moreover, there was a slight but significant increase in renal blood flow (significant at 30, 60–90, and 120 min), whereas the reductions in superior mesenteric and hindquarter flows observed in the vehicle-treated group were absent in the presence of isradipine (0.05 mg·kg⁻¹·min⁻¹) (Fig. 1B). The maximum increases in renal flow (+26 ± 6%) were reached 90 min after the start of insulin infusion. Furthermore, in the isradipine (0.05 mg·kg⁻¹·min⁻¹)-treated group, there was a significant increase in renal vascular conductance (significant at 15–30, 75–90, and 120 min) but no consistent effect on superior mesenteric or hindquarter vascular conductances, when compared with the effects of control infusion of saline-0.2% BSA (Fig. 2B). The maximum increase in renal vascular conductance (+32 ± 8%) was observed 120 min after the euglycemic infusion of insulin was begun. These responses were significantly different from those in vehicle-receiving group in which euglycemic infusion of insulin had no effect on renal vascular conductance but produced a marked reduction in superior mesenteric and hindquarter vascular conductances.

In the groups of rats treated with the high dose of isradipine (0.15 mg·kg⁻¹·min⁻¹), we found that the euglycemic infusion of insulin had no effect on heart rate or mean blood pressure (Fig. 1B). However, there was a late but significant increase in renal blood flow (significant at 30 and 75–120 min), compared with measurements after a control infusion of saline-0.2% BSA (Fig. 1B). The maximum rise in renal blood flow was +47 ± 12% and occurred 120 min after the beginning of insulin infusion. The latter response was significantly different from that seen in the group not...
receiving isradipine in which euglycemic infusion of insulin had no effect on renal blood flow. Isradipine did not influence the reduction in superior mesenteric flow previously observed in the untreated group, although this effect was not significant in the isradipine-treated animals. Moreover, the reduction in hindquarter flow observed in the group of rats not receiving isradipine was abolished by the treatment with isradipine (Fig. 1B). These cardiovascular responses to euglycemic infusion of insulin in the presence of isradipine (0.15 mg·kg\(^{-1}\)·min\(^{-1}\)) were associated with a rise in renal vascular conductance (significant at 30 and 90–120 min), but no significant change in hindquarter vascular conductance was found (Fig. 2B). The maximum increase in renal vascular conductance (+59 ± 13%) was observed 120 min after the start of insulin infusion. These vascular responses differed significantly from those seen in the untreated group, in which insulin had no effect on renal vascular conductance, but produced a significant reduction in hindquarter vascular conductance. The superior mesenteric vasoconstrictor response observed previously in the rats not receiving isradipine was not significantly influenced by isradipine, although this effect did not reach the level of significance in the group of rats treated with isradipine (0.15 mg·kg\(^{-1}\)·min\(^{-1}\); Fig. 2B).

Responses During Euglycemic-Hyperinsulinemic Clamp

Figure 3 shows that, in the fasting state, basal arterial plasma glucose levels, as well as mean plasma glucose in the second hour of the euglycemic-hyperinsulinemic clamp, were slightly but significantly higher in both groups of rats treated with isradipine than in the untreated group. However, there was no difference in basal arterial plasma insulin levels among the groups. Moreover, during the euglycemic-hyperinsulinemic clamp period, fasting plasma insulin levels in the three groups of rats rose acutely and achieved similar plateaus, whereas normal plasma glucose levels were maintained in the three groups of rats. However, the glucose-infusion rate required to maintain euglycemia during the last hour of the clamp, the conditions of which closely approximated a steady-state insulin concentration and that represented the whole body glucose utilization, was significantly higher in both groups of isradipine treated rats than in the rats receiving 15% DMSO.

Effect of Hydralazine

Resting values for cardiovascular variables measured before and 60 min after the beginning of a
continuous intravenous infusion of hydralazine in conscious SHR are shown in Table 2. Thus the infusion of hydralazine caused a significant decrease in basal mean blood pressure values but caused no changes in basal heart rate values. These changes were accompanied by significant increases in renal, superior mesenteric, and hindquarter vascular conductances.

In the group of rats receiving a continuous infusion of saline (n = 11) instead of hydralazine, we found that the euglycemic infusion of insulin at the dose of 4 mU·kg⁻¹·min⁻¹ caused cardiovascular changes that were similar to those previously observed in the first series of experiments. Thus there were no effects on heart rate, mean blood pressure, or renal blood flow, whereas slight but significant decreases in superior mesenteric (−14 ± 2%) and hindquarter (−17 ± 6%) flows were observed. Moreover, there were significant falls in superior mesenteric (−17 ± 3%) and hindquarter (−21 ± 6%) vascular conductances but no change in renal vascular conductance.

In the group of rats receiving hydralazine, we found that euglycemic infusion of insulin (4 mU·kg⁻¹·min⁻¹) caused slight but significant increases in heart rate (significant at 45–120 min) and mean blood pressure (significant at 30–60 and 90 min), when compared with the effects of control infusion of saline-0.2% BSA (Fig. 4). Moreover, there was a significant increase in renal blood flow (significant at 45–90 min and 120 min), whereas the reductions in superior mesenteric and hindquarter flows observed in the vehicle-treated group were absent in the presence of hydralazine (Fig. 4). The maximum increases in heart rate (+63 ± 12%), mean blood pressure (+18 ± 4%), and renal flow (+17 ± 7%) were reached 90, 60, and 75 min, respectively, after the start of insulin infusion. Furthermore, in the hydralazine-treated group, there was a significant decrease in superior mesenteric vascular conductance (significant at 30–120 min), but no consistent effect on renal or hindquarter vascular conductances, when compared with the effects of control infusion of saline-0.2% BSA (Fig. 5). The maximum decrease in superior mesenteric vascular conductance (−14 ± 8%) was observed 75 min after beginning the euglycemic infusion of insulin. These responses were not significantly different from those in vehicle-receiving group in which euglycemic infusion of insulin had no effect on renal vascular conductance but produced a marked reduction in superior mesenteric and hindquarter vascular conductances.

Table 2. Cardiovascular effects of iv infusion of hydralazine in conscious, spontaneously hypertensive rats

<table>
<thead>
<tr>
<th></th>
<th>HR, beats/min</th>
<th>MAP, mmHg</th>
<th>Doppler Shift, kHz</th>
<th>Vascular Conductance, (kHz/mmHg) x 10³</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Renal</td>
<td>Mesenteric</td>
</tr>
<tr>
<td></td>
<td>321 ± 9</td>
<td>325 ± 11</td>
<td>6.7 ± 0.7</td>
<td>12.1 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>134 ± 3</td>
<td>106 ± 4*</td>
<td>7.4 ± 0.7*</td>
<td>13.1 ± 1.3</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 17 rats. Before, cardiovascular variables measured in resting rats before any drug treatment. After, same cardiovascular variables measured 60 min after the beginning of a continuous iv infusion of hydralazine (0.25 mg·kg⁻¹·h⁻¹). HR, heart rate; MAP, mean arterial blood pressure. *P < 0.05 vs. Before, Student’s t-test for paired data.
Responses During Euglycemic-Hyperinsulinemic Clamp Performed in the Presence of Hydralazine

Table 3 shows that, in the fasting state, basal arterial plasma glucose and insulin levels, as well as mean plasma glucose in the second hour of the euglycemic-hyperinsulinemic clamp, were similar in both groups of rats, the untreated group and the group receiving hydralazine. Moreover, during the euglycemic-hyperinsulinemic clamp period, fasting plasma insulin levels in both groups rose acutely and achieved similar plateaus, whereas normal plasma glucose levels were maintained in both groups of rats. However, the average glucose infusion rate required to maintain euglycemia by 10.2 ± 0.3 mg·kg⁻¹·h⁻¹ on June 22, 2017 http://ajpendo.physiology.org/ Downloaded from

| Vehicle | 5.4 ± 0.2 | 5.0 ± 0.2 | 29.6 ± 5.5 | 677.3 ± 38.8 | 20.3 ± 1.5 |
| Hydralazine | 5.7 ± 0.3 | 5.4 ± 0.2 | 23.4 ± 4.0 | 586.2 ± 37.4 | 31.3 ± 1.7* |

Values are means ± SE. Euglycemic-hyperinsulinemic clamp has been performed by infusing regular porcine insulin at a rate of 4 mU·kg⁻¹·min⁻¹. GIR60–120, glucose infusion rate required to maintain euglycemia during steady-state (60–120 min) plasma insulin concentration. *P < 0.05 hydralazine (0.25 mg·kg⁻¹·h⁻¹)-treated rats (n = 8) vs. vehicle (saline; n = 11), Student’s t-test for unpaired data.
during the last hour of the clamp (GIR\textsubscript{60–120}) was significantly higher in the group of hydralazine-treated rats than in the rats not receiving hydralazine.

**DISCUSSION**

One of the principal findings of this study is that short-term treatment with the calcium channel antagonist isradipine in conscious SHR can lower blood pressure and markedly improve insulin sensitivity, as evident from the significant difference in the insulin-sensitivity index between the isradipine-treated groups and the group not receiving isradipine. Improvement in insulin sensitivity has also been reported in obese and lean nondiabetic hypertensive subjects after treatment with some antihypertensive agents, such as \(\beta\)-blockers (38), converting enzyme inhibitors (37), and calcium channel antagonists (4, 22), although there have been conflicting reports with the latter (5, 28). Calcium channel antagonists are a fairly new group of drugs that are increasingly used in antihypertensive treatment. However, the information on the effects of calcium channel antagonists on glucose metabolism or insulin sensitivity is very scanty and often contradictory. Hence, a slight deterioration in glucose tolerance (19) as well as diabetogenic effects (5) has been reported during treatment with nifedipine, whereas with some other calcium channel antagonists, glucose tolerance or insulin sensitivity were unchanged (15, 28) and even improved (4, 8, 22). Thus it seems likely that different calcium channel antagonists may have different effects on carbohydrate regulation. However, part of the discrepancy might result from the dose of the antagonist used, because in vitro studies have demonstrated that insulin-mediated cellular glucose uptake is optimal only within a certain range of intracellular calcium concentrations, with higher or lower calcium levels leading to impaired glucose tolerance and insulin sensitivity (12).

Consistent with our results, a recent study carried out in conscious SHR has shown that the calcium channel antagonist nitrendipine improves glucose tolerance by increasing muscle glucose uptake (8). However, a previous study comprising 11 type II diabetic patients treated with isradipine (29) showed that isradipine has no effect on glucose tolerance, insulin secretion, and insulin action. Although the diabetic population evaluated in that study showed slight elevations in blood pressure, the latter were considered in the normal range and the antihypertensive effect of isradipine was less striking than in patients with frank hypertension. Therefore, we cannot exclude different results in patients with severe hypertension, in that isradipine could be more effective in partially reversing insulin resistance in nondiabetic hypertensive subjects. Thus the effects of calcium channel antagonists on carbohydrate regulation might differ, depending on the basal metabolic state. In relation with this latter remark, another point needs to be discussed. The SHR used in the present study were 12–14 wk old, a time at which blood pressure is significantly higher in SHR than in Wistar-Kyoto rats (36), the normotensive reference strain, and at which resistance to insulin modulation of glucose metabolism (36) and vascular reactivity (32, 36) have been demonstrated. However, the levels of hypertension found in these rats were not very high (i.e., mean arterial blood pressure: 134 mmHg), suggesting that the rats might not have developed their full expression of high blood pressure. Therefore, we cannot exclude the possibility that greater effect would have been observed with isradipine if the level of hypertension would have been more pronounced as in older SHR.

Considering the mechanism underlying the positive effect we observed on glucose metabolism in isradipine-treated SHR, our results strongly suggest that part of the improvement in the insulin sensitivity may be related to hemodynamic factors, as previously suggested by Bursztyn et al. (8) in nitrendipine-treated SHR. As seen in the present study, a short-term treatment with isradipine caused a significant decrease in blood pressure. This effect was accompanied by significant vasodilation in renal, superior mesenteric, and hindquarter vascular beds in rats treated with the low dose of isradipine. Several studies have reported a positive correlation between blood flow in skeletal muscle and insulin sensitivity (3, 25, 26, 31). Moreover, skeletal muscle has been identified as the main site of glucose uptake (2) and the primary site of insulin resistance in essential hypertension (9) and in SHR (23). Because glucose and transcapillary insulin deliveries to tissue have been reported as important modulators of skeletal muscle glucose uptake (41, 47), it is likely that isradipine may exert the observed beneficial effects through its hemodynamic actions (thereby increasing blood flow to skeletal muscle, which may lead to increased delivery of glucose and insulin to muscle and enhanced nonoxidative pathways of glucose disposal; Ref. 3).

On the other hand, human studies have also indicated that insulin resistance could be related, at least in part, to an inability of insulin to appropriately increase skeletal muscle blood flow (3). A vasodilator response to insulin has been reported in humans (1, 3, 25, 31), conscious dogs (34), and rats (36). It has been suggested that, through its vasodilator action, insulin could enhance its own ability to promote glucose uptake into skeletal muscle, by increasing the distribution of insulin and glucose to a greater mass of previously underperfused muscle (3, 31). Impairment in the insulin-mediated skeletal muscle vasodilator response has been shown in states of insulin resistance, such as obesity, type II diabetes, and hypertension (3, 30, 31). In a recent study, we found that the euglycemic infusion of insulin at a rate of 4 mU·kg\(^{-1}\)·min\(^{-1}\) in normotensive Wistar-Kyoto rats caused significant vasodilation in renal and hindquarter vascular beds but no changes in blood pressure, heart rate, or superior mesenteric vascular conductance (36). In contrast to Wistar-Kyoto rats, and similarly to what we found in the present study, we observed that the same infusion of insulin in SHR had no effect on heart rate, blood pressure, or renal vascular conductance but caused significant vaso-
constriction in the superior mesenteric and hindquarter vascular beds (36). Therefore, the physiological effect of insulin to vasodilate skeletal muscle and renal vasculature is impaired in SHR. Moreover, we demonstrated a reduction in the insulin-sensitivity index in the SHR compared with the Wistar-Kyoto rats (36). In the present study, we found significant improvement in the insulin-sensitivity index in both groups of isradipine-treated rats, and we observed also major differences in the cardiovascular responses to euglycemic infusion of insulin between the isradipine-treated groups and the group of rats not receiving isradipine. In the isradipine-treated groups there were significant renal vasodilator responses to insulin, whereas the superior mesenteric and hindquarter vasoconstrictor responses observed in the untreated group were completely abolished in both groups of isradipine-treated rats. Therefore, the inhibition of the vasoconstrictor responses to insulin in the latter may have contributed to improve insulin and glucose distribution to insulin-sensitive tissues and then to enhance the insulin action on glucose metabolism.

Another potential mechanism by which isradipine could improve insulin sensitivity, might be related to its cytosolic free calcium lowering effect, as previously reported with other calcium channel antagonists (12, 45). Calcium channel antagonists are arterial vasodilators that reduce the cytosolic calcium content mainly through a reduction of transmembrane calcium influx in vascular smooth muscle cells. According to some studies, insulin resistance in hypertension could be attributed, at least in part, to the elevated intracellular calcium level (33, 42). Hence, part of the beneficial effect of isradipine on insulin sensitivity may have resulted from a reduction of cytosolic free calcium concentrations toward normal levels. To test whether the acute effects of isradipine were due to hemodynamic factors or were caused by reduction in intracellular calcium concentration, we carried out experiments with hydralazine, as a positive vasodilator control. Thus, similarly to what we observed with isradipine, we found that short-term treatment with hydralazine in conscious SHR lowers blood pressure and markedly improves insulin sensitivity, as evident from the significant difference in the insulin-sensitivity index between the hydralazine-treated group and the group not receiving hydralazine. The blood pressure lowering effect of hydralazine was accompanied by marked increases in blood flow and vasodilation in renal, superior mesenteric, and hindquarter vascular beds. However, except for the heart rate response we observed in the hydralazine-treated group, we found that the cardiovascular responses to euglycemic infusion of insulin were not significantly different from those in vehicle-receiving group. Thus it is likely that the improvement we observed on glucose metabolism in hydralazine-treated SHR was related to the hemodynamic changes elicited by hydralazine. Furthermore, these results suggest that the beneficial effects of isradipine are not specific to reduction in intracellular calcium but would rather result from the vasodilator effects of isradipine.

Although the present findings support the hemodynamic link between insulin resistance and hypertension, some authors have questioned as to whether SHR are in fact an insulin-resistant model (7). Indeed, the SHR are often heterogeneous with regard to glucose metabolism (27). However, in a previous study with a similar protocol, we found a clear and significant decrease in the insulin-sensitivity index in SHR compared with their age-matched normotensive control, the Wistar-Kyoto rats (36). Moreover, the insulin-sensitivity index (as determined by the glucose infusion rate during the last hour of the clamp) found in the present study for the untreated rats (17.3 ± 1.7 mg·kg⁻¹·min⁻¹) was similar to that we previously measured in the SHR (16.9 ± 1.9 mg·kg⁻¹·min⁻¹; Ref. 36).

Treatments with isradipine were associated with modest but not significant decreases in fasting plasma insulin levels in both groups of rats, whereas slight but significant increases in fasting plasma glucose levels were observed in both groups of isradipine-treated rats. Significant elevation of glucose levels and reduction of insulin secretion have also been reported in humans during an oral glucose tolerance test after short-term oral or high-dose intravenous infusions of calcium channel antagonist (10). However, fasting and postcarbohydrate load plasma glucose or insulin levels have often been reported not to change during long-term treatment with various calcium channel antagonists (44). Thus, as the mechanisms that control insulin secretion (46) and insulin-mediated cellular glucose uptake (12) depend at least in part on cytosolic calcium concentration, it is likely that depending on the dose and the type of calcium channel antagonist, as well as the condition of the treatment (short-term vs. long-term treatment), different effects might be observed on fasting plasma insulin and glucose levels.

In conclusion, the present data indicate that the calcium channel antagonist isradipine significantly reduces blood pressure and improves insulin sensitivity in SHR presumably secondary to hemodynamic factors. This finding is supported by experiments with hydralazine as a positive vasodilator control. Moreover, short-term treatment with isradipine was found to abolish the superior mesenteric and hindquarter vasoconstrictor responses to euglycemic infusion of insulin, which may have contributed to improve insulin and glucose distribution and to enhance the insulin-mediated cellular glucose uptake in insulin-sensitive tissues. Thus the present study further supports the hemodynamic concept of insulin resistance in hypertension and suggests that vasodilator agents may be useful drugs for these conditions.

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