Effect of metformin on the vascular and glucose metabolic actions of insulin in hypertensive rats

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We investigated the long-term effect of metformin treatment on blood pressure, insulin sensitivity, and vascular responses to insulin in conscious spontaneously hypertensive rats (SHR). The rats were instrumented with intravascular catheters and pulsed Doppler flow probes to measure blood pressure, heart rate, and blood flow. Insulin sensitivity was assessed by the euglycemic hyperinsulinemic clamp technique. Two groups of SHR received metformin (100 or 300 mg·kg⁻¹·day⁻¹) for 3 wk while another group of SHR and a group of Wistar Kyoto (WKY) rats were left untreated. We found that vasodilation of skeletal muscle and renal vasculatures by insulin is impaired in SHR. Moreover, a reduced insulin sensitivity was detected in vivo and in vitro in isolated soleus and extensor digitorum longus muscles from SHR compared with WKY rats. Three weeks of treatment with metformin improves the whole-body insulin-mediated glucose disposal in SHR but has no blood pressure-lowering effect and no influence on vascular responses to insulin (4 mU·kg⁻¹·min⁻¹). An improvement in insulin-mediated glucose transport activity was detected in isolated muscles from metformin-treated SHR, but in the absence of insulin no changes in basal glucose transport activity were observed. It is suggested that part of the beneficial effect of metformin on insulin resistance results from a potentiation of the hormone-stimulating effect on glucose transport in peripheral tissues (mainly skeletal muscle). The results argue against a significant antihypertensive or vascular effect of metformin in SHR.

Santuré, Marta, Maryse Pitre, Nathalie Gaudreault, André Marette, André Nadeau, and Hélène Bachelard. Effect of metformin on the vascular and glucose metabolic actions of insulin in hypertensive rats. Am J Physiol Gastrointest Liver Physiol 278: G682–G692, 2000.—We investigated the long-term effect of metformin treatment on blood pressure, insulin sensitivity, and vascular responses to insulin in conscious spontaneously hypertensive rats (SHR). The rats were instrumented with intravascular catheters and pulsed Doppler flow probes to measure blood pressure, heart rate, and blood flow. Insulin sensitivity was assessed by the euglycemic hyperinsulinemic clamp technique. Two groups of SHR received metformin (100 or 300 mg·kg⁻¹·day⁻¹) for 3 wk while another group of SHR and a group of Wistar Kyoto (WKY) rats were left untreated. We found that vasodilation of skeletal muscle and renal vasculatures by insulin is impaired in SHR. Moreover, a reduced insulin sensitivity was detected in vivo and in vitro in isolated soleus and extensor digitorum longus muscles from SHR compared with WKY rats. Three weeks of treatment with metformin improves the whole-body insulin-mediated glucose disposal in SHR but has no blood pressure-lowering effect and no influence on vascular responses to insulin (4 mU·kg⁻¹·min⁻¹). An improvement in insulin-mediated glucose transport activity was detected in isolated muscles from metformin-treated SHR, but in the absence of insulin no changes in basal glucose transport activity were observed. It is suggested that part of the beneficial effect of metformin on insulin resistance results from a potentiation of the hormone-stimulating effect on glucose transport in peripheral tissues (mainly skeletal muscle). The results argue against a significant antihypertensive or vascular effect of metformin in SHR.

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A VARIETY OF ORALLY ACTIVE antihyperglycemic agents are frequently used to help manage the glucose intolerance and insulin resistance of patients with type II diabetes. Some of these agents that increase insulin sensitivity and decrease plasma insulin levels have been shown to lower blood pressure in humans and attenuate the development of experimental hypertension in both genetic and acquired models of hypertension. Metformin, a biguanide, has been shown to improve insulin resistance and, in some studies, to lower blood pressure in hypertensive patients. Particularly relevant is the study by Landin et al. (19), in which metformin treatment of nonobese, nondiabetic untreated hypertensives led to an improvement in insulin sensitivity and reduction in blood pressure that was reversed after cessation of treatment. Similar results were found in a group of obese, hypertensive women (11) and obese patients with type II diabetes in which the reduction of blood pressure was closely related to the antihyperglycemic effect (12). In spontaneously hypertensive rats (SHR) and fructose-fed Sprague-Dawley rats, but not in normotensive Wistar Kyoto (WKY) rats or normal Sprague-Dawley rats, chronic treatment with metformin has been shown to decrease hyperinsulinemia, improve insulin sensitivity, and prevent the development of hypertension (32, 33). Furthermore, it has been demonstrated that the antihypertensive effect of metformin can be reversed when plasma insulin levels are increased to the levels seen before treatment (32, 33). The mechanism underlying the blood pressure-lowering effect of metformin remains obscure and largely controversial (6, 13, 29, 30).

So far, no attention has been paid to the vascular effects of metformin in conscious, unrestrained animals and on the possibility that important regional hemodynamic changes induced by long-term treatment with metformin contribute to the antihypertensive effect reported in some studies and possibly also to the improvement in insulin sensitivity (by increasing insulin and glucose deliveries to insulin-sensitive tissues). Therefore, the present study was designed to investigate the effect of long-term treatment (3 wk) with metformin on blood pressure, plasma insulin levels, insulin sensitivity, and regional hemodynamic responses to insulin in SHR. The rats were chronically instrumented with intravascular catheters and pulsed Doppler flow probes to permit a continuous recording of blood pressure, heart rate, and regional blood flow. Insulin sensitivity was assessed by using the euglycemic hyperinsulinemic clamp technique in conscious unrestrained rats, as well as by measuring glucose uptake rates in isolated skeletal muscles in which insulin’s hemodynamic effects are no longer apparent.

METHODS

All surgical and experimental procedures followed institutional animal care guidelines. Male SHR and WKY rats (aged 12–14 wk and weighing 200–300 g) were purchased from

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Three separate groups of SHR and one group of WKY rats were used in this study. The first group of SHR (n = 8) received metformin at a dose of 100 mg·kg<sup>-1</sup>·day<sup>-1</sup>, and a second group of SHR (n = 12) received metformin at a dose of 300 mg·kg<sup>-1</sup>·day<sup>-1</sup>. Metformin was provided in drinking water for 3 wk, and the solution was freshly prepared every day. The two other groups of rats [one group of SHR (n = 25) and one group of WKY rats (n = 19)] did not receive metformin but were treated in the same way as the metformin groups and served as a control for the drug treatment groups. Two weeks after the beginning of the treatment, the rats were anesthetized with a mixture of ketamine and xylazine (100 and 10 mg/kg ip, respectively, supplemented as required) and had pulsed Doppler flow probes implanted to monitor changes in renal, mesenteric, and hindquarter blood flows, according to the method previously developed by Gardiner and Bennett (9) and as previously described in detail (24). At least seven days later, the rats were reanesthetized with the same mixture of ketamine and xylazine. The leads of the implanted probes were soldered to a microconnector (Microtech), two separate catheters were implanted in the right jugular vein (for glucose and insulin infusions), 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. After a further 48-h recovery, experiments were begun in conscious unrestrained animals with free access to water but not food.

Throughout the experiments, continuous recordings were made of phasic and mean blood pressures, instantaneous heart rate, and phasic and mean renal, mesenteric, and hindquarter blood flows, according to the method previously developed by Gardiner and Bennett (9) and as previously described in detail (24). At least seven days later, the rats were reanesthetized with the same mixture of ketamine and xylazine. The leads of the implanted probes were soldered to a microconnector (Microtech), two separate catheters were implanted in the right jugular vein (for glucose and insulin infusions), 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. After a further 48-h recovery, experiments were begun in conscious unrestrained animals with free access to water but not food.

Experimental Protocols

Euglycemic hyperinsulinemic clamp studies. The rats were deprived of food for 12-14 h overnight before the glucose clamp study was begun. 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 sedated rats. The euglycemic hyperinsulinemic clamp was then carried out over 2 h while heart rate, blood pressure, and regional blood flows were measured continuously. Therefore, hemodynamic and insulin sensitivity measurements were done simultaneously in the same rats. Each rat received a continuous infusion of regular porcine insulin (Iletin II, 100 U/ml; Eli Lilly, Indianapolis, IN) at rate of 4 mU·kg<sup>-1</sup>·min<sup>-1</sup>. The insulin solution was diluted to the appropriate concentration in saline (0.9% NaCl) containing 0.2% BSA to prevent the adsorption of insulin by the glassware and plastic surfaces. Insulin was infused using 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 infusion range of 20 µl/min at the end of the tubing. In control experiments, subgroups of untreated SHR (n = 10) and WKY rats (n = 9) were infused with saline-0.2% BSA (20 µl/min) instead of insulin to match approximately the saline load delivered during the clamp studies. Five minutes after the insulin infusion was begun, a 30% 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). 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 corresponds to the steady-state concentration of insulin, was used as an index of insulin sensitivity. Blood samples (0.3 ml) were collected before the beginning of the clamp and at timed (20 min) intervals during 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. At the end of the clamp, food was returned to the rats. Two days later, the rats were deprived again of food for 12 h and new experiments were carried out to measure glucose transport activity in isolated skeletal muscles.

Glucose transport activity in isolated rat skeletal muscles. The effect of chronic treatment with metformin on basal and insulin-stimulated glucose utilization was examined in isolated soleus and extensor digitorum longus (EDL) skeletal muscles from both treated groups and compared with that observed in the untreated SHR group. Basal and insulin-stimulated glucose transport activity were also measured in isolated skeletal muscles (soleus and EDL) from WKY rats and compared with that seen in the untreated SHR. Glucose transport in isolated muscles was measured by use of the glucose analog [3H]2-deoxy-D-glucose as described previously (17). The rats were anesthetized with a mixture of ketamine and xylazine (100 and 10 mg/kg ip, respectively). Soleus and EDL muscles were dissected out and rapidly cut into 20- to 30-mg strips and incubated for 30 min at 30°C in a shaking water bath into 25 ml flasks containing 3 ml of oxygenated Krebs-Ringer bicarbonate (KRB) buffer supplemented with 8 mM glucose, 32 mM mannitol, and 0.1% BSA (RIA grade). Flasks were gassed continuously with 95% O<sub>2</sub>-5% CO<sub>2</sub> throughout the experiment. After the initial incubation, muscles were incubated for 30 min in oxygenated KRB buffer in the absence or presence of insulin (Humulin R) at four different concentrations (0.002, 0.02, 0.2, and 2 µU/ml). Muscles were then washed for 10 min at 29°C in 3 ml of KRB buffer containing 40 mM mannitol and 0.1% BSA. Muscles were then incubated for 20 min at 29°C in 3 ml KRB buffer containing 0.25, 0.5, and 1.0 mM [3H]2-deoxy-D-glucose (2.25 µCi/ml), 32 mM [14C]mannitol (0.3 µCi/ml), 2 mM sodium pyruvate, and 0.1% BSA. Insulin was present throughout the wash and uptake incubations (if it was present in the previous incubation medium). After the incubation, muscles were rapidly blotted at 4°C, clamp frozen, and stored at −80°C until processed. Muscles were processed by boiling for 10 min in 1 ml of water. Extracts were transferred to an ice bath, vortexed, and then centrifuged at 1,000 g. Triplicate 200-µ1 aliquots of the muscle extract supernatant and of the incubation medium were counted for radioactivity using a Wallac 1409 counter. [3H]2-deoxy-D-glucose uptake rates were corrected for extracellular trapping using [14C]mannitol (15).
Analytical 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 (27) using a glucose analyzer (Technicon RA-XT), and plasma insulin level was measured by RIA using porcine insulin standards and polyethylene glycol for separation (5).

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

RESULTS

Resting values for cardiovascular variables in the four groups of rats are shown in Table 1. Long-term treatment with metformin, at a dose of 100 or 300 mg·kg⁻¹·day⁻¹, had no effect on basal mean arterial blood pressure, heart rate, or renal or hindquarter blood flows or vascular conductances when compared with measurements in untreated SHR. However, we found a slightly higher basal superior mesenteric blood flow in SHR treated with metformin (at low dose only) than in untreated SHR, but there was no difference in basal superior mesenteric vascular conductance. As expected, the basal mean arterial blood pressure in WKY rats was lower than in SHR. This was accompanied by lower basal heart rate and higher basal renal blood flow, although there was no significant difference in basal superior mesenteric or hindquarter flows between SHR and WKY rats. Moreover, we found higher basal renal vascular conductance in WKY rats than in SHR, but similar basal superior mesenteric and hindquarter vascular conductances in both strains.

Hemodynamic Responses to Insulin Infusion During the Euglycemic Hyperinsulinemic Clamp Period

In the group of SHR rats not treated with metformin (n = 15), we found that euglycemic infusion of insulin at the dose of 4 mU·kg⁻¹·min⁻¹ had no effect on heart rate, whereas a slight but significant increase in mean arterial blood pressure was observed (significant at 30–60 and 90–120 min) compared with the effects of control infusion of saline-0.2% BSA (Fig. 1A). Moreover, there were no changes in renal or hindquarter blood flows but a significant decrease in superior mesenteric blood flow (significant at 15–120 min; Fig. 1A). The maximum increase in mean arterial blood pressure (9 ± 5 mmHg) and the maximum decrease in superior mesenteric blood flow (−22 ± 5%) were achieved 45 and 90 min, respectively, after the start of insulin infusion. Furthermore, in the SHR group, there was a significant decrease in superior mesenteric vascular conductance (significant at 15–120 min) but no consistent effect on renal or hindquarter vascular conductances, compared with the effects of control infusion of saline-0.2% BSA (Fig. 2A). The maximum decrease in superior mesenteric vascular conductance (−23 ± 7%) was reached 90 min after the start of insulin infusion. Thus, in summary, there was a pressor response accompanied by significant decreases in superior mesenteric blood flow and vascular conductance following euglycemic infusion of insulin in SHR.

In both groups of metformin-treated rats, we found that the euglycemic infusion of insulin at a dose of 4 mU·kg⁻¹·min⁻¹ produced cardiovascular effects that were not significantly different from the untreated SHR (Figs. 1B and 2B). Thus, in the group of rats chronically treated with metformin at the dose of 100 mg·kg⁻¹·day⁻¹ (n = 8), we observed significant increases in blood pressure (significant at 30–120 min) and decreases in superior mesenteric blood flow (significant at 15, 45–60, and 90–120 min) but no changes in heart rate or hindquarter blood flow 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 30–75 and 105 min). However, the latter response was not significantly different from that seen in untreated SHR, in which euglycemic infusion of insulin had no effect on renal blood flow. The maximum increases in mean arterial blood pressure (13 ± 3 mmHg) and renal blood flow (15 ± 4%) were reached 105 and 60 min after the start of insulin infusion, respectively. The maximum decrease in superior mesenteric blood flow (−12 ± 5%) was observed 45 min after the start of insulin infusion. These changes in

Table 1. Baseline values of heart rate, mean arterial blood pressure, regional Doppler shift, and regional vascular conductance in conscious, unrestrained rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>HR, bpm</th>
<th>MAP, mmHg</th>
<th>Doppler Shift, kHz</th>
<th>Vascular Conductance, kHz·mmHg·10⁻³</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Renal</td>
<td>Mesenteric</td>
</tr>
<tr>
<td>SHR</td>
<td>25</td>
<td>335 ± 7</td>
<td>135 ± 2</td>
<td>6.0 ± 0.6</td>
<td>10.7 ± 1.4</td>
</tr>
<tr>
<td>Metformin-SHR, 100 mg·kg⁻¹·day⁻¹</td>
<td>8</td>
<td>325 ± 8</td>
<td>137 ± 5</td>
<td>6.3 ± 0.6</td>
<td>14.2 ± 1.0*</td>
</tr>
<tr>
<td>Metformin-SHR, 300 mg·kg⁻¹·day⁻¹</td>
<td>12</td>
<td>318 ± 9</td>
<td>142 ± 3</td>
<td>7.2 ± 0.8</td>
<td>15.1 ± 1.6</td>
</tr>
<tr>
<td>WKY</td>
<td>19</td>
<td>316 ± 8*</td>
<td>97 ± 3*</td>
<td>8.1 ± 0.6*</td>
<td>8.7 ± 0.9</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of rats. Groups represent those used to assess hemodynamic effects of intravenously infused insulin during euglycemic hyperinsulinemic clamp studies. Comparisons were made between spontaneously hypertensive rats (SHR) and metformin-treated SHR, as well as between SHR and Wistar Kyoto (WKY) rats. HR, heart rate; MAP, mean arterial pressure. *P < 0.05 vs. SHR by Student’s t-test for unpaired data.
blood pressure and blood flows were accompanied by significant decreases in superior mesenteric vascular conductance (significant at 15 and 45–120 min) but no changes in renal or hindquarter vascular conductances (Fig. 2B). The maximum decrease in superior mesenteric vascular conductance (−15 ± 5%) was reached 90 min after the beginning of the infusion of insulin.

Similarly, in the group of rats chronically treated with metformin at a dose of 300 mg·kg⁻¹·day⁻¹ (n = 12), we found that the euglycemic infusion of insulin caused significant increases in blood pressure (significant at 30–120 min) and decreases in superior mesenteric blood flow (significant at 15, 45, 60, and 105–120 min) and vascular conductance (15–120 min) but no changes in heart rate, renal or hindquarter blood flows, or renal vascular conductance compared with the effects of control infusion of saline-0.2% BSA (Figs. 1B and 2B). There was a significant decrease in hindquarter vascular conductance (significant at 30–120 min). However, this response was not significantly different from that seen in untreated rats, in which euglycemic infusion of insulin had no effect in this vascular bed. The maximum changes in mean arterial blood pressure (MAP) and decreases in superior mesenteric blood flow were observed among the 3 groups of rats. HR, heart rate; bpm, beats per minute. *P < 0.05 for insulin-infused group vs. vehicle-infused group by ANOVA followed by Fisher’s test.
been observed in both metformin-treated and untreated SHR.

In WKY rats \((n = 10)\), the same euglycemic infusion of insulin had no effect on mean blood pressure, whereas a slight increase in heart rate (significant at 75–120 min) and a late but significant increase in renal flow (significant at 90–120 min) were observed when compared with the effects of control infusion of saline-0.2% BSA (Fig. 3). The maximum rises in heart rate (22 ± 8 bpm) and renal blood flow (16 ± 4 %) occurred 105 and 90 min, respectively, after the beginning of insulin infusion. These findings were not different from those observed in untreated SHR, although in the latter there was no increase in heart rate or renal blood flow but a slight pressor response. However, in WKY rats no change in superior mesenteric flows but a long-lasting increase in hindquarter flow (significant at 15–120 min) was observed following the infusion of insulin. The maximum rise in hindquarter flow was 21 ± 5 % and was achieved 60 min after beginning the insulin infusion. These responses differed significantly from those seen in untreated SHR, in which the same infusion of insulin produced significant decreases in superior mesenteric vascular conductance but produced no change in renal and hindquarter vascular conductances. In summary, the present results indicate that euglycemic infusion of insulin in WKY rats causes significant increases in heart rate and renal and hindquarter blood flows and vascular conductances. Except for the heart rate and renal blood flow effects, these cardiovascular changes were found to be significantly different from those seen in SHR.

Responses During Euglycemic Hyperinsulinemic Clamp

Figure 5 shows that, in the fasting state, basal arterial blood glucose levels were similar in the three groups of SHR rats. Moreover, there was no difference in basal arterial plasma insulin levels between the untreated SHR and the group of rats treated with both doses of metformin. During the euglycemic hyperinsulinemic clamp period, the plasma insulin concentration in the three groups of rats rose acutely to a similar plateau and blood glucose levels were held constant (Fig. 5). However, the average glucose infusion rate required to maintain euglycemia during the last hour of the clamp, the conditions of which closely approxi-

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**Fig. 2.** Changes in regional vascular conductances elicited by intravenous infusion of saline-0.2% BSA \((\circ, n = 10)\) or euglycemic infusion of insulin at a rate of 4 mU·kg\(^{-1}\)·min\(^{-1}\) in conscious, untreated SHR \((A, \bullet, n = 15)\) or SHR chronically treated with metformin at a dose of 100 mg·kg\(^{-1}\)·day\(^{-1}\) \((B, \triangle, n = 8)\) or 300 mg·kg\(^{-1}\)·day\(^{-1}\) \((B, \downarrow, n = 12)\). Data were derived from data shown in Fig. 1. Effects of vehicle or insulin were assessed relative to baseline values. Values are means ± SE. Similar decreases in superior mesenteric vascular conductance were observed among the 3 groups of rats.*\(P < 0.05\) for insulin-infused group vs. vehicle-infused group by ANOVA followed by Fisher’s test.
A steady-state insulin concentration and which represented the whole body glucose utilization, was significantly higher in both groups of metformin-treated rats than in the untreated SHR group. A slightly but significantly higher basal level of blood glucose was noted in WKY rats than in the untreated SHR group, whereas no difference was found in basal arterial plasma insulin levels between both strains (Fig. 6). During the euglycemic hyperinsulinemic clamp period, the plasma insulin concentration in the two groups of rats rose acutely to a similar plateau and blood glucose levels were held constant. Blood glucose levels during the euglycemic-hyperinsulinemic clamp were slightly but significantly higher in the group of WKY rats than in the untreated SHR. The average glucose infusion rate required to maintain euglycemia during the last hour of the clamp was found to be

\[ \frac{50}{20} \]
significantly higher in the group of WKY rats than in the untreated SHR group (Fig. 6).

Effect of a Chronic Treatment with Metformin on \([\text{H}]2\)-deoxy-o-glucose Uptake in Isolated Skeletal Muscles

The effect of long-term treatment with metformin on basal and insulin-stimulated glucose uptake in isolated soleus and EDL muscles is shown in Fig. 7. In the isolated soleus muscle, we found that metformin, at doses of 100 and 300 mg·kg\(^{-1}\)·day\(^{-1}\), had no influence on basal glucose transport compared with that observed in untreated SHR (Fig. 7A). However, in the presence of low doses of insulin (i.e., 0.002 mU/ml for the group of rats treated with 300 mg·kg\(^{-1}\)·day\(^{-1}\) metformin and 0.02 mU/ml for both groups of rats treated with metformin), we found a significant increase in insulin-activated glucose transport compared with those measured in untreated rats. These differences were no longer observed in the presence of higher doses of insulin (i.e., 0.2 and 2 mU/ml).

Figure 7B shows that long-term treatment with metformin at a dose of 100 mg·kg\(^{-1}\)·day\(^{-1}\) had no effect on basal or insulin-stimulated glucose transport (at any doses of insulin tested) in the isolated EDL muscle compared with that measured in untreated SHR. However, in the isolated EDL from rats treated with the high dose of metformin, we found a clear and significant increase in insulin-stimulated glucose transport at the doses of 0.002, 0.02, and 0.2 mU/ml. Again, no significant effects of metformin were observed at the highest dose of insulin.

Figure 8 shows that the basal glucose transport activity measured in soleus and EDL muscles isolated from WKY rats was not different from that seen in untreated SHR. However, at doses of 0.002 and 0.02
mU/ml of insulin, we found a significantly higher insulin-stimulated glucose transport activity in soleus muscles isolated from WKY rats than in those isolated from SHR (Fig. 8A). Moreover, in the EDL muscles isolated from WKY rats, we found a significantly higher insulin-stimulated glucose transport activity (at all doses of insulin tested) than in those isolated from SHR (Fig. 8B).

DISCUSSION

Metformin and Insulin Sensitivity

The present study indicates that at the whole animal level long-term treatment with metformin, at doses of 100 and 300 mg·kg\(^{-1}\)·day\(^{-1}\), significantly improves insulin sensitivity in SHR. Although there are very few published studies on metformin effects on glucose metabolism in nondiabetic hypertensive subjects, the present results agree with previous findings demonstrating that chronic treatment with metformin improves insulin sensitivity in normoglycemic insulin-resistant hypertensive patients (6, 19), in nondiabetic hypertensive subjects (29), and in a group of obese nondiabetic hypertensive women (11). Moreover, recent studies carried out in SHR and fructose-fed rats demonstrated that long-term treatment (over 12 wk) with metformin (at a dose of 500 mg·kg\(^{-1}\)·day\(^{-1}\)) started before the development of hypertension prevented the increase in plasma insulin levels (32, 33). The SHR used in the present study were 12–14 wk old, a time at which there is fixed hypertension. Moreover, according to the experiments carried out with their age-matched normotensive controls, the WKY rats, we found a significantly lower insulin sensitivity index in SHR rats, which confirms our previous finding (24) and agrees with other studies indicating that SHR are insulin resistant (25, 26, 31).

Metformin and Glucose Uptake in Muscles

In the present study, we examined the effect of long-term treatment with metformin on glucose transport in isolated SHR skeletal muscles. The soleus and EDL muscles were obtained from untreated control SHR and WKY rats and from metformin-treated SHR.

Fig. 7. Effect of chronic treatment with metformin on insulin dose-response curve for stimulation of glucose uptake in (A) soleus muscle and (B) extensor digitorum longus (EDL) muscle. Muscles were dissected out from untreated SHR (○, n = 6) and SHR chronically treated with metformin at dose of 100 mg·kg\(^{-1}\)·day\(^{-1}\) (△, n = 5) and 300 mg·kg\(^{-1}\)·day\(^{-1}\) (▲, n = 5). Values are means ± SE. *P < 0.05 for group of rats treated with high dose of metformin vs. untreated group by Student’s t-test for unpaired data. †P < 0.05 for group of rats treated with low dose of metformin vs. untreated group by Student’s t-test for unpaired data.

Fig. 8. Insulin dose-response curve for stimulation of glucose uptake in soleus muscle (A) and EDL muscle (B). Muscles were dissected out from Wistar Kyoto rats (○, n = 5) and SHR (●, n = 6). Values are means ± SE. Comparisons were made between insulin-evoked responses in Sprague Dawley rats and those in Wistar rats. *P < 0.05 Wistar Kyoto rats vs. SHR by Student’s t-test for unpaired data.
The nonmetabolizable glucose analog [3H]2-deoxy-D-glucose was used in the present study, thereby evaluating the glucose uptake process separately. Moreover, since no metformin was added to the incubation medium, the results primarily reflect the effects of three weeks of exposure of the tissue to metformin. Thus, our results show that skeletal muscles isolated from untreated SHR are less sensitive to the insulin-stimulating effect on glucose transport compared with WKY rats. Three weeks of treatment with metformin was found to have a potentiating effect on insulin-stimulated glucose transport activity in both soleus and EDL muscles from SHR without affecting the basal rate of glucose uptake. These results are consistent with previous findings demonstrating that metformin treatment enhanced the effects of insulin on glucose uptake in insulin-resistant human skeletal muscle (8) and in skeletal muscle isolated from streptozotocin diabetic mice (1, 20) and alloxan diabetic rats (7) but had no effect in the absence of insulin (8, 21). The precise mechanism for the stimulating effect of metformin on skeletal muscle glucose transport cannot be assessed from the prevailing data. However, previous studies have indicated that insulin binding was not affected by the presence of metformin in soleus muscles from diabetic mice (20) despite an increase in insulin-stimulated glycogenesis, suggesting that metformin exerts its major action at the postreceptor level. Thus metformin could possibly act by altering the intrinsic activity of the glucose transporters at the plasma membrane or by recruiting more transporters to the plasma membrane from an intracellular pool (18, 22). However, some recent studies do not support this concept (28) and suggest that metformin would act at a step distal to that of glucose transport (14).

Metformin and Blood Pressure

The available evidence provides conflicting results regarding the effect of metformin on blood pressure in humans and rodents. Improvement in insulin sensitivity with parallel reduction in blood pressure have been reported in nonobese nondiabetic untreated hypertensive patients (19), in obese diabetic and nondiabetic hypertensive women (11, 12), and in normotensive patients with type II diabetes (4). Studies on SHR and fructose-fed Sprague-Dawley rats have shown that long-term treatment with metformin before the development of hypertension prevented the development of hyperinsulinemia and attenuated the increase in systolic blood pressure and that these effects are correlated (32, 33). However, in several of these studies, metformin was also associated with a decrease in body weight, raising the possibility that the antihypertensive effect was secondary to weight loss. In hypertensive patients, weight reduction can be associated with lowering of blood pressure (23), although this is not always the case (16).

In contrast, several studies have failed to demonstrate an antihypertensive effect of metformin in obese and nonobese insulin-resistant normoglycemic hypertensive patients (6, 13), in nondiabetic hypertensive patients (29, 30), and in experimental models of hypertension (34). In agreement with those studies, the present results indicate that three weeks of treatment with metformin does not decrease blood pressure in conscious, unrestrained SHR. Interestingly, we have been unable to demonstrate any significant vascular changes following treatment with metformin. Together, these results argue against a significant antihypertensive effect of metformin in hypertensive subjects and rats. The reason for the conflicting results of the effect of metformin on blood pressure is not readily apparent. However, a number of differences in experimental design and procedures may account for the contradictory findings. Thus differences in the group population (e.g., the initial insulin sensitivity and the severity of hypertension), the dose of metformin used, as well as the duration of the treatment may contribute to the different results.

Metformin and Insulin-Mediated Hemodynamic Responses

In WKY rats, the euglycemic infusion of insulin causes vasodilations in renal and hindquarter vascular beds but no changes in mean blood pressure or superior mesenteric vascular conductance. In contrast, in SHR the same dose of insulin was found to produce a slight increase in mean blood pressure and a marked vasoconstriction in superior mesenteric vascular bed. Therefore, the vasodilation of skeletal muscle and renal vasculatures by insulin is impaired in SHR. Impairment in the vascular response to insulin is thought to contribute to deficient uptake of glucose by peripheral tissues (presumably due to less delivery of glucose) (2, 3). Thus it was the goal of the present study to examine the possibility that part of the beneficial effect of metformin on insulin resistance might be attributed to some regional hemodynamic changes or influences on the hemodynamic responses to insulin following long-term treatment with metformin. The results indicate that three weeks of treatment with metformin has no hemodynamic effect and has no influence on the insulin-mediated vasconstrictor and pressor responses observed in the SHR. Although the present study is limited to measurements of total blood flow into skeletal muscle beds and does not address whether changes in blood flow distribution occur within the muscle, it is suggested that metformin treatment improves insulin sensitivity in SHR by a mechanism that is in some way dependent on hemodynamic factors or on the vascular responses to insulin, but that possibly involves a potentiation of insulin action on glucose extraction at the level of skeletal muscle, which accounts for most of the peripheral glucose use. This is consistent at least with the higher insulin-stimulated glucose transport response we noted in skeletal muscles isolated from SHR treated with metformin.

In summary, major differences in cardiovascular responses to insulin between SHR and WKY rats were observed. Mainly, the vasodilation of skeletal muscle and renal vasculatures by insulin was impaired in SHR. Moreover, the SHR were found to be significantly impaired in skeletal muscle and renal vasculatures by insulin was impaired in SHR.
less sensitive to insulin action than WKY rats. Three weeks of treatment with metformin significantly improved whole-body insulin-mediated glucose disposal in SHR but had no statistically significant effect on basal blood pressure or regional vascular conductances and had no influence on cardiovascular responses to insulin in conscious SHR. In preparations of isolated soleus and EDL muscles, we found that metformin treatment in SHR improved the insulin-mediated glucose transport activity, particularly at low concentrations of insulin, whereas in the absence of insulin no changes in basal glucose transport activity were observed in either muscle. Therefore, the present results are consistent with previous findings indicating that metformin treatment improves insulin sensitivity in hypertensive and insulin-resistant rats and suggest that part of its beneficial action on insulin resistance results from a potentiation of the hormone-stimulating effect on glucose transport in peripheral tissues (mainly skeletal muscle). The results argue against a significant antihypertensive or vascular effect of metformin in SHR.

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