Metformin Attenuates Salt-Induced Hypertension in Spontaneously Hypertensive Rats

Martin S. Muntzel, Ibrahima Hamidou, Shean Barrett

Abstract—Metformin, an antihyperglycemic agent used for treatment of type 2 diabetes mellitus, lowers blood pressure in humans and experimental animals. We recently demonstrated that short-term administration of metformin may lower blood pressure by reducing sympathetic neural outflow. The present studies were initiated to determine whether long-term administration of metformin blunts salt-induced hypertension, a condition characterized by elevated sympathetic activity. Male spontaneously hypertensive rats, in which radioeleceters had been implanted for continuous monitoring of heart rate and blood pressure, were randomly assigned to groups that received vehicle (drinking water) or metformin (500 mg/kg per day) and ate a normal 0.3% NaCl diet and to groups that received vehicle or metformin and ate a high 8.0% NaCl diet for a period of 4 weeks. Although metformin did not affect blood pressure in the animals that ate the normal-salt diet (vehicle, 130±3 mm Hg; metformin, 133±5 mm Hg; mean±SEM), drug treatment blunted the rise in pressure caused by a high-salt diet (vehicle, 153±4 mm Hg; metformin, 140±5 mm Hg; P<0.001). In agreement, during direct pressure recordings in anesthetized rats, the animals that ate the high-salt diet had higher pressures (136±13 mm Hg) than those in the control (98±5 mm Hg, P<0.01), metformin (100±7 mm Hg, P<0.01), and metformin/high-salt groups (92±3 mm Hg, P<0.01). Finally, metformin lowered heart rate in rats that ate the normal- and high-salt diets (310±3 and 305±4 bpm) compared with rats that ate normal- and high-salt diets given vehicle (332±3 and 324±2 bpm, P<0.01). These data indicate that the chronic depressor actions of metformin are enhanced in animals with hypertension exacerbated by a high-salt diet. (Hypertension. 1999;33:1135-1140.)

Key Words: metformin ▪ rats, inbred SHR ▪ blood pressure ▪ heart rate ▪ sodium chloride, dietary

Metformin is an antihyperglycemic agent used for the treatment of type 2 diabetes mellitus. In clinical trials, metformin has been shown to increase insulin sensitivity and to effectively lower blood glucose levels without increasing plasma insulin.1 In addition to its salutary effects on glucose metabolism, metformin lowers blood pressure in certain human subjects2–5 but not in others.6–9 Although the drug also reduces blood pressure in experimental animals,10–14 the physiological conditions of blood pressure lowering are unclear.

Earlier studies that examined the relationship between insulin resistance and hypertension have demonstrated simultaneous lowering of plasma insulin and blood pressure by metformin in both humans and experimental animals.2,3,10,11,15 These results led to the hypothesis that metformin reduces blood pressure by lowering insulin. Recent observations, however, suggest a direct action of metformin on vascular smooth muscle. For example, incubation of arterial ring segments in metformin reduced intracellular calcium transients to contractile agents and caused reductions in contraction strength.16–18 As an additional mechanism of blood pressure reduction, we found that α-adrenergic blockade or ganglionic blockade abolished acute depressor responses to intravenous metformin in spontaneously hypertensive rats (SHRs), which suggests a sympathoinhibitory action of the drug.12

In previous work, SHRs were found to be particularly susceptible to the hypertensive actions of high dietary NaCl19–21. The increase in pressure is secondary, in part, to elevations in sympathetic nerve activity.19–21 Given the evidence supporting a sympathoinhibitory role of metformin, we predicted that the drug would exert powerful antihypertensive actions in SHRs challenged by NaCl loading. We initiated the present studies to establish whether chronic metformin would lower blood pressure in SHRs and to determine whether this antihypertensive effect is enhanced in animals consuming an 8.0% NaCl diet. Previous studies with metformin may have been less accurate because they used restraint to determine tail-cuff systolic blood pressures. Therefore, a second aim was to use implantable transmitter devices to examine continuous blood pressure and heart rate (HR) effects in freely moving animals.

Methods

Animals

Male SHRs, weighing 175 to 200 g, were purchased from Taconic Laboratories (Germantown, NY). The animals were housed individ-
Metformin and Dietary NaCl

ually in polycarbonate cages on pine-shaving bedding in a temperature-controlled colony room illuminated on a 12/12 light/dark cycle. All procedures were approved by the Lehman College Institutional Animal Care and Use Committee and were performed in accordance with the Lehman College and National Institutes of Health guidelines for the care and use of experimental animals.

Implantation and Use of Radiotelemetry Devices

Rats were anesthetized by subcutaneous injection with ketamine (40 mg/kg IM) supplemented with xylazine (5 mg/kg IM) and given antibiotic (penicillin, 60,000 IU IM). The animals were then prepared for surgery with the use of sterile techniques. In brief, a midline incision was made and the abdominal aorta was carefully exposed caudally to the renal arteries. The tip of the catheter from the radiotelemetry device (model TA11PA-C40, Data Sciences International) was inserted through a hole made by a 21-gauge needle, and the catheter was then fixed to the artery with cyanoacrylate cement (Vetbond, 3 M) and a fiber patch so as not to occlude the flow of blood.

After surgery, rats were placed individually in cages and blood pressure was recorded continuously with a radiotelemetry recording system, which consisted of the implanted radiotelemetry device, a receiver (model RLA1020, Data Sciences), and a calibrated pressure analog adapter (model R11CPA, Data Sciences) connected to a MacLab data acquisition system (ADI Instruments, Milford, Mass) interfaced with a Macintosh computer. HR was calculated from the blood pressure pulse with the use of the MacLab system.

Experimental Protocol

After a week of habituation to their new environment, the rats underwent surgery for implantation of the radiotelemetry devices. The animals were allowed 1 week of postsurgery recovery, followed by a week-long baseline period, during which body weight, water intake, HR, and blood pressure were monitored daily. At the end of the baseline period, the rats were randomly assigned to 4 experimental groups: a control group (n = 6) that received water and ate an 8% NaCl diet (Harlan-Teklad, Madison, Wis), a high-salt group (n = 6) that received water and ate an 8% NaCl diet, a metformin group (n = 6) that received metformin in the drinking water and ate a 0.3% NaCl diet (Harlan-Teklad), a metformin/high-salt group (n = 6) that received metformin in the drinking water and ate an 8% NaCl diet (Harlan-Teklad), a metformin group (n = 8) that received metformin in the drinking water and ate a 0.3% NaCl diet, and a metformin/high-salt group (n = 6) that received metformin in the drinking water and ate an 8% NaCl diet. Treatment with metformin was initiated at a dose of 350 mg/kg per day and was increased to 700 mg/kg per day in the metformin/high-salt group. Treatment with metformin was initiated at a dose of 350 mg/kg per day and was increased to 700 mg/kg per day in the metformin/high-salt group. Treatment with metformin was initiated at a dose of 350 mg/kg per day and was increased to 700 mg/kg per day in the metformin/high-salt group.

Because previous studies with metformin examined tail-cuff systolic pressures,10,11,13,14 we addressed the possibility that the drug may affect blood pressure differentially during restraint. To test this, we measured 5 minutes of unrestrained blood pressure between 1200 and 1500 hours and then immediately placed the rats in acrylic length-adjustable restrainers, which are typically used for the measurement of tail-cuff pressures, and recorded restrained blood pressures for an additional 5 minutes. This procedure was performed during the second week after initiation of the experimental regimens and again at the end of the study.

At completion of the study, the accuracy of the telemetry system was verified in anesthetized rats (ketamine and xylazine) by simultaneously measuring daytime blood pressures (1000 to 1500 hours) from the radiotelemeter and from a catheter placed in the left femoral artery. Pressures from the catheter were taken with a Statham P23 XL pressure transducer and displayed on a Grass model 7E polygraph (Astro-Med Inc).

Data Analysis

Because 24-hour recordings of HR and mean arterial pressure (MAP) reveal diurnal oscillations, with values increasing at night and decreasing during the day, separate analyses were made for the 12-hour day periods, 12-hour night periods, and entire 24-hour periods. In addition, we analyzed HR and MAP during the last hours of the light period (1600 to 1800 hours) to characterize the sponta-

    Figure 1. Effect of metformin and high dietary salt on 24-hour MAP during 34 days of study. Observations were made on day 1 and every other day thereafter. Values are mean ± SEM. * indicates P<0.05 vs control and metformin groups; and †, P<0.05 vs high-salt group.

Results

Radiotelemetry Blood Pressure

Twenty-four-hour MAP values, shown in Figure 1, reveal normal developmental increases in blood pressure in control SHRs over the 34 days of study. Blood pressure was not altered by metformin in SHRs that consumed a normal 0.3% NaCl diet. As expected, introduction of the 8.0% NaCl diet elicited rapid and sustained elevations in MAP compared with the control and metformin-treated groups (P<0.001). However, treatment with metformin largely attenuated the salt-induced rise in blood pressure, which produced intermediate blood pressure values. As a result, SHRs in the metformin/high-salt group had significantly lower MAPs than high-salt rats (P<0.001) yet higher pressures than control and metformin-treated animals (P<0.001).

Because blood pressure values during the entire experiment showed marked diurnal oscillations, we analyzed MAP values separately for the 12-hour day and 12-hour night periods (Table 1). Although nighttime MAP revealed the same pattern of results observed during the 24-hour analysis, daytime MAPs failed to show significantly lower pressures in metformin/high-salt rats compared with high-salt animals. In other words, metformin significantly attenuated salt-induced hypertension during the night but not during the day. This was explained by the finding that high salt potentiates the awakening rise in MAP during nighttime onset, whereas metformin attenuates this rise (Figure 2).
Radiotelemetry HR
The 24-hour recording of HR showed overall decreases in all groups, which reflects a gradual habituation to the experimental conditions (P<0.001; Figure 3). HR values were not different between the control and high-salt SHRs. In contrast, metformin caused a rapid and long-lasting decrease in HR in both metformin-treated groups. Because the magnitude of the bradycardia was slightly larger in the metformin/high-salt group, these animals had a significantly lower HR than control and high-salt SHRs (P<0.01), whereas the metformin group had lower values compared with the control group only (P<0.01).

As with blood pressure, HR increased during the night and decreased during the day. In contrast with the pattern of results observed for blood pressure, the awakening rise in HR was not affected by high dietary salt. Treatment with metformin, however, reduced awakening HR increases, as evidenced by significantly lower increases in both metformin groups compared with the control group (Figure 2; P<0.05).

Direct Recording of MAP
Comparison of simultaneous direct recordings of blood pressure with radiotelemetry values in anesthetized rats showed no differences between the 2 methods. Interestingly, under anesthetized conditions, metformin completely abolished the rise in blood pressure caused by high dietary salt (Figure 4).

Blood Pressure Responses to Restraint Stress
During the second week of the study, increases in MAP in response to restraint were equivalent in the control (166±6 mm Hg), high-salt (165±6 mm Hg), metformin (173±7 mm Hg), and metformin/high-salt (170±5 mm Hg) groups. When restraint was presented at study completion, overall blood pressure increases were lower in magnitude but again did not differ between the control (158±9 mm Hg), high-salt (153±9 mm Hg), metformin (153±6 mm Hg), and metformin/high-salt (155±6 mm Hg) groups.

### Table 1. Effect of Dietary Salt and Metformin on Average 12-Hours-Light, 12-Hours-Dark, and 24-hour Values of MAP and HR at End of Experimental Period

<table>
<thead>
<tr>
<th>Group</th>
<th>MAP, mm Hg</th>
<th>HR, bpm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12 h Light</td>
<td>12 h Dark</td>
</tr>
<tr>
<td>Control</td>
<td>122±2</td>
<td>129±3</td>
</tr>
<tr>
<td>High salt</td>
<td>141±3*</td>
<td>153±3†</td>
</tr>
<tr>
<td>Metformin</td>
<td>126±4</td>
<td>132±4</td>
</tr>
<tr>
<td>Metformin/high salt</td>
<td>133±6*</td>
<td>141±5†</td>
</tr>
</tbody>
</table>

End-of-study MAPs, HRs, and SEMs were averaged over 3 12-h-light, 3 12-h-dark, and 3 24-h periods from days 29, 31, and 33. Values are group mean±SEM.

*P<0.05 vs control and metformin groups.
†P<0.001 vs all other groups.
‡P<0.01 vs control and high-salt groups.
§P<0.01 vs control group.

**Figure 2.** Effect of metformin and high dietary salt on the awakening rise in MAP and HR. The awakening rise is defined as the spontaneous increase in MAP and HR during the last 2 hours of the light cycle (1600 to 1800 hours). Values are mean±SEM. § indicates P<0.05 vs control and metformin groups; and †, P<0.05 vs control group.

**Figure 3.** Effect of metformin and high dietary salt on 24-hour HR during 34 days of study. Values are mean±SEM. # indicates P<0.05 vs control group; and §, P<0.05 vs control and high-salt groups.
Body Weight, Fluid Intake, and Metformin Intake

Metformin decreased body weight by ≈30 g in both the metformin and metformin/high-salt groups (Table 2). The overall ANOVA demonstrated a group-by-repeated-measures interaction (*P < 0.01), which indicates equivalent weight gains during the baseline period followed by slower gains in the metformin groups. Fluid intakes reflected the level of salt in diets (Table 2). The ANOVA for fluid intake showed a group-by-repeated-measures interaction (*P < 0.001), which indicates similar intakes during the baseline period followed by greatly increased fluid ingestion in the 2 high-salt groups. Although fluid intakes differed greatly between groups, the concentrations of metformin were adjusted to deliver equivalent amounts to the metformin (average of days 29 to 33, 598 ± 62 mg/kg per day) and metformin/high-salt (average of days 29 to 33, 561 ± 58 mg/kg per day) groups.

Discussion

There is increasing evidence that the antidiabetic drug metformin lowers blood pressure under certain physiological conditions.2–5,10–14 Major new results in the current study were that metformin strongly attenuated the rise in blood pressure caused by high dietary salt, and this rise was abolished when blood pressure was measured in anesthetized rats. Metformin also produced long-lasting decreases in HR that were independent of dietary NaCl level.

The finding that metformin, by itself, did not affect blood pressure in SHRs was unexpected. Other groups have reported hypotensive actions of the drug given to SHRs at the same dose and route as in the present study10 or as a smaller dose administered subcutaneously.14 Because these experiments determined blood pressure with the use of the tail plethysmographic method in restrained rats, metformin may produce relatively lower blood pressures under conditions of restraint. Confinement stress, however, elicited similar blood pressure increases in all groups. As a more likely explanation, we initiated metformin in SHRs that weighed 230 g, whereas others began administration when the rats weighed ≈100 g10 or at 6 to 8 weeks of age (≈120 to 200 g).14 Which raises the possibility that the drug attenuates hypertension development in young SHRs but has little effect in adult animals.

The mechanism of metformin-induced attenuation of salt-induced hypertension may involve interaction with the sympathetic nervous system. Although NaCl loading and concomitant volume expansion normally elicit homeostatic decreases in sympathetic activity,22 SHRs exhibit increases in plasma and urinary norepinephrine and exaggerated depressor responses to ganglionic blockade.19–21 These results suggest that NaCl loading elevates sympathetic nerve activity in SHRs and that metformin may attenuate this elevation. Supporting a sympathoinhibitory role of metformin, acute injections caused decreases in blood pressure and renal sympathetic nerve activity that were abolished by ganglionic blockade.12,23 In addition, long-term administration decreased plasma norepinephrine in experimental animals,13 as well as in certain clinical trials,4 but not in others.7,24,25 Although our observation of metformin-induced bradycardia is consistent with decreased sympathetic drive, it is impossible to rule out drug-induced elevations in parasympathetic activity.

In the current experiment, metformin attenuated salt-induced hypertension during the active period at night but failed to significantly lower pressure during the daytime, when the animals were resting. Because increases in blood pressure at night correspond with elevations in locomotor activity in rats,26 the nighttime attenuation in blood pressure may be secondary to metformin-induced sedation. Although we cannot rule out this possibility, recent studies have failed to demonstrate metformin-induced reductions in locomotor activity in Wistar rats.27 The nighttime attenuation in blood pressure may also be related to the feeding patterns of rats. Because rats feed and drink at night, the acute hypotensive actions of metformin, administered in the drinking water, would be expected to be most pronounced during that period.

Related to the finding that metformin attenuated nighttime blood pressure was our observation of a surge in pressure on awakening that was enhanced by high dietary salt but attenuated in metformin/high-salt SHRs (Figure 2). Salt-induced increases in awakening blood pressure have been reported previously for SHRs.28 Also, in SHRs, the awaken-

![Figure 4. Direct MAPs from arterial catheters in anesthetized rats at completion of the study. Values are mean ± SEM. § indicates *P < 0.01 vs all other groups.](http://hyper.ahajournals.org/)

**Table 2. Effect of Dietary Salt and Metformin on Body Weight and Fluid Intake**

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Weight, g</th>
<th>Fluid Intake, ml/kg per d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>End of Study</td>
</tr>
<tr>
<td>Control</td>
<td>232 ± 4</td>
<td>313 ± 5</td>
</tr>
<tr>
<td>High salt</td>
<td>239 ± 5</td>
<td>307 ± 8</td>
</tr>
<tr>
<td>Metformin</td>
<td>231 ± 5</td>
<td>279 ± 4†</td>
</tr>
<tr>
<td>Metformin/high</td>
<td>226 ± 6</td>
<td>270 ± 7†</td>
</tr>
</tbody>
</table>

End-of-study values represent the average of group means and SEM from days 29, 31, and 33. All other values are mean ± SEM.

*P < 0.01 vs control and metformin groups.
†P < 0.01 vs control and high-salt groups.
ing surge was attenuated by prazosin and clonidine yet not affected by captopril or hydralazine, which had similar antihypertensive efficacy, which suggests that these elevations are due to α-adrenoceptor-mediated vasoconstriction as a result of sympathetic activation. Therefore, our observation that metformin attenuates the awakening surge in high-salt SHRs supports the hypothesis that the drug blunts salt-induced sympathoexcititation.

Another mechanism of blood pressure reduction involves metformin-induced attenuation of fluid volume expansion. Salt-induced elevations in vascular fluid volume and cardiac output may be reduced by metformin because the drug increases urinary excretion of sodium and other cations through elevations in glomerular filtration rate without affecting renal tubular transport. Finally, metformin may attenuate salt-induced hypertension by lowering body weight or decreasing insulin. However, in the current study, equivalent weight reductions in normal-salt/metformin–treated rats were not associated with alterations in blood pressure. Furthermore, previous studies in rats showed metformin-induced reductions in blood pressure before decreases in weight were noted, and clinical trials demonstrated reduced blood pressures without changes in body weight or body mass index, which suggests that weight reduction is not necessary for blood pressure lowering. Supporting a mechanism involving plasma insulin, studies in animals and humans have shown metformin-induced reductions in both insulin and blood pressure.

In summary, long-term treatment with metformin reduced HR in adult SHRs but lowered blood pressure only in animals with hypertension exacerbated by high dietary NaCl. Therefore, some aspect of salt-induced hypertension, such as increased sympathetic nerve activity or volume expansion, was blunted by metformin treatment, which caused attenuated blood pressure increases. Whether metformin chronically affects sympathetic activity or renal function remains to be determined.

Acknowledgments

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