Nitric Oxide Opposes Glucose-Induced Hypertension by Suppressing Sympathetic Activity

Christie R. Claxton, Michael W. Brands

Abstract—We have reported that glucose infusion in L-NAME–treated rats increased arterial pressure more than the additive responses to glucose and L-NAME alone. This suggested that nitric oxide synthesis inhibition potentiated the hypertensive response to chronic glucose infusion, and the heart rate data suggested an important role for the sympathetic nervous system. This study tested the role of the sympathetic nervous system by infusing glucose for 7 days in 4 groups of rats: L-NAME (L), L-NAME plus α- and β-adrenergic receptor blockade (LB), vehicle, or vehicle plus adrenergic receptor blockade (blockers). Mean arterial pressure (MAP, 24 hours per day) increased significantly in both the vehicle and blockers groups, confirming our previous reports. Likewise, MAP increased significantly more during glucose infusion in the L rats, from 120±3 mm Hg to 158±4 mm Hg by day 7, which was >3 times the increase in the vehicle rats. Heart rate also increased significantly in the L rats, from 391±4 to 426±8 bpm, and that increase was prevented completely in the LB rats. However, although the increase in MAP in the LB rats was significantly less than in the L rats, the hypertension was not prevented completely. The explanation for that partial inhibition is not clear, but the overall effectiveness of adrenergic receptor blockade to attenuate the potentiated hypertensive and tachycardic responses to glucose infusion in the L-NAME–treated rats versus the normal rats suggests that nitric oxide may help protect against hypertension during glucose infusion through suppression of sympathetic activity. (Hypertension. 2003; 41:274-278.)

Key Words: glucose ■ blood pressure ■ nitric oxide ■ sympathetic nervous system ■ heart rate

Chronic intake of diets high in simple sugars such as fructose and glucose are known to cause insulin resistance, hyperinsulinemia, and hypertension.1–7 Several systems have been shown to play significant roles in mediating the hypertensive response, such as angiotensin II,3,8 thromboxane,7,9 and endothelin10; however, the role of the nitric oxide system is not clear. This is due in part to the reported effect of insulin to stimulate nitric oxide production and cause vasodilatation11,12 but of decreased endothelial-dependent vasodilation to play an important role in the hypertensive response.4 Thus, under conditions of hyperinsulinemic hyperglycemia, such as in type II diabetes or with sugar loading, it is not clear whether nitric oxide is protecting against the development of hypertension or whether gradual impairment of the system is a mechanism for causing the hypertension. There probably is an element of both, but our recent results13 support an important role for the former.

We have reported that a 7-day glucose infusion in rats increases mean arterial pressure (MAP) significantly5,13 and that that effect is exacerbated markedly in rats with chronic inhibition of nitric oxide synthesis.13 This is consistent with the observation of Bursztyn et al14 that a subpressor dose of L-NAME uncovered a hypertensive response to hyperinsulinemia. However, those authors infused insulin subcutaneously and without sugar supplementation, whereas we induced hyperinsulinemia through a glucose infusion. This model, in fact, has been shown to replicate all the measured characteristics of our earlier insulin plus glucose infusion model8,9,15–17 suggesting that the ultimate level of hyperinsulinemia in those studies was driven by the glucose infusion and also strongly linking those studies with the experimental models that achieve glucose loading through dietary supplementation (eg, fructose feeding). However, although we saw no evidence for a role of the sympathetic nervous system in normal rats,17 the ~50 bpm increase in heart rate we measured in the L-NAME–treated, hypertensive rats during glucose infusion13 suggested that the sympathetic nervous system played an important role under those conditions. The present study was designed to test that hypothesis.

Methods

All experiments were conducted in male Sprague-Dawley rats (~350 g, Harlan Sprague-Dawley, Madison, Wis), and the protocols were approved by the Institutional Animal Care and Use Committee. Chronic artery and vein catheters were implanted under aseptic surgical conditions and connected to a dual-channel hydraulic swivel after recovery, as described previously.13 The rats were housed in individual metabolic cages, and sodium intake throughout the experiment was maintained constant at ~2.9 mmol/d by continuous
intravenous infusion of 18 mL per day sterile, 0.9% saline, combined with sodium-deficient rat chow, as described previously. In addition, 19 mL of sterile water was infused as vehicle for the glucose infusion. This infusion was begun immediately after placement of the rats in the metabolic cages, and 5 to 7 days were allowed for acclimation before control measurements were recorded.

**Experimental Protocol**

Rats were assigned randomly to 4 groups that received glucose infusion plus (1) L-NAME (L, n=8), (2) L-NAME plus α1- and β-adrenergic receptor blockade (LB, n=9), (3) vehicle (n=6), or (4) vehicle plus α1- and β-adrenergic receptor blockade (blockers, n=6). After determining that the rats were in sodium balance and that MAP was stable, L-NAME (10 μg/kg per minute) was added to the intravenous infusate in the L and LB rats, and α1- and β-adrenergic receptor blockade was begun in the LB and blockers rats, using terazosin at 6 mg/kg per day and propranolol at 10 mg/kg per day, respectively. Those infusions were maintained for the remainder of the experiment. Six days were allowed for the control period, then a 7-day glucose infusion was begun in all groups by replacing the sterile water vehicle from the control infusate with 50% dextrose solution that provided 18.5 mg glucose/kg per minute. The experimental period lasted 7 days and was followed by a recovery period with removal of glucose in all groups.

On the fifth day of the control, the third day of the experimental, and the end of the recovery periods, arterial blood (1.3 mL) was collected from the fasted rats in chilled sodium EDTA tubes for measurement of plasma insulin concentration and renin activity (PRA), blood glucose concentration, and for gamma counting. The sample was replaced with an equal volume of saline.

**Analytical Methods**

Glomerular filtration rate was measured by calculating the clearance of 125I-in-octamethyl (Glofil) after 24-hour infusion of the isotope, as described previously. Plasma insulin was measured with a Merodia insulin ELISA kit (ALPCO) with rat standards. PRA was measured by radioimmunoassay, and blood glucose was measured with an Accuchek III blood glucose analyzer. Urinary sodium and potassium concentrations were determined with the use of ion-sensitive electrodes (Nova). The effectiveness of the α1- and β-receptor blockade during the study was assessed by analyzing the MAP responses to bolus infusions of the α1- and β-receptor agonists phenylephrine (4 μg IV) and isoproterenol (0.70 μg IV), respectively. Results are presented as mean±SEM. Data were analyzed with a repeated-measures ANOVA, and within-group comparisons against control data were made with the Dunnett test, with the use of SAS Statview software. With a significant between-group F test, specific between-group differences were identified with completely randomized ANOVAs and Fisher least significant difference testing for each day. Statistical significance was considered to be *P*<0.05.

**Results**

Mean arterial pressure increased in the vehicle group during the 7-day glucose infusion as reported previously; however, the rise was statistically significant only on day 3 of glucose infusion (Figure 1). Also as described previously, MAP increased significantly during glucose infusion in the blockers rats, and Figure 2 shows that the change in pressure was not different between the vehicle and the blockers rats. Thus, chronic adrenergic receptor blockade did not have a significant effect on the blood pressure response to glucose infusion in normal rats. Chronic L-NAME infusion, however, markedly potentiated the effect of glucose infusion to increase MAP, as we have reported previously, and that effect was attenuated significantly by adrenergic receptor blockade. L-NAME treatment increased baseline MAP significantly from 91±2 to 120±3 mm Hg, and glucose infusion caused a rapid increase to 136±3 mm Hg on day 1 and a progression to 158±4 mm Hg by day 7 (Figure 1). Adrenergic receptor blockade attenuated baseline L-NAME hypertension, although not significantly, to an average of 109±3 mm Hg in the LB rats before glucose infusion (Figure 1). The difference in MAP between the LB and L groups increased during glucose infusion, as MAP increased significantly in the LB rats to an average of 133±4 mm Hg by day 7, which was significantly less than MAP in the L rats, both in absolute pressure (Figure 1) and when expressed as change in pressure (Figure 2). Thus, the potentiation of glucose hypertension by L-NAME depended significantly on adrenergic mechanisms.

Adrenergic receptor blockade decreased baseline heart rate significantly from 419±14 bpm to an average of 365±11 bpm before glucose infusion in the blockers rats (Figure 1), and glucose infusion increased heart rate to an average of 374±4 bpm, although this was not significant statistically. Baseline heart rate decreased similarly in the LB rats after adrenergic blocker treatment, and Figure 1 shows that glucose infusion increased heart rate significantly in that group. Figure 2 shows that the change in heart rate in the two adrenergic blockade groups during glucose was almost superimposable. In contrast to those modest increases, and the virtually flat heart rate response to glucose in the vehicle group, heart rate increased by ~35 bpm in the L rats during glucose infusion, from an average of 391±4 to an average of 426±8 bpm. This response to adrenergic receptor blockade in the LB rats is consistent with a major role for the sympathetic nervous system in the response to glucose infusion in rats with chronic L-NAME treatment.
Consistent with our previous reports, PRA decreased significantly during glucose infusion in the vehicle rats but increased progressively and significantly during glucose infusion in the L-NAME–treated rats (Figure 3). Treatment with adrenergic receptor blockers decreased baseline PRA significantly in vehicle rats (blockers versus vehicle) but not in L-NAME rats, in which baseline PRA already was decreased (LB versus L); however, adrenergic receptor blockade did prevent the progressive rise in PRA from occurring during glucose infusion in the L-NAME–treated rats (LB versus L). Glomerular filtration rate during the control period averaged 2.9±0.2 and 2.9±0.1 mL/min in the L and LB groups, respectively, and 3.0±0.3 and 3.7±0.6 mL/min in the vehicle and blockers groups, respectively. Glomerular filtration rate decreased significantly during glucose infusion, as we have reported previously, and there were no differences in the response between any group.

There also were no significant differences in the change in the plasma insulin or glucose responses to glucose infusion in any of the groups. Baseline insulin appeared to be increased in the LB group, but the probability value for the between-group F test from the ANOVA was 0.21, reflecting the variability in the glucose and recovery period measurements as well. However, there was a significant increase in insulin in all groups of a magnitude consistent with our previous reports. Glucose increased significantly in all groups and returned to control during the recovery period. There were no significant changes in plasma protein concentration or hematocrit in any group during the experiment. The blood pressure responses to bolus infusions of phenylephrine and isoproterenol were measured before and during chronic α1- and β-adrenergic receptor blockade, and we achieved >85% inhibition of the respective pressor and depressor responses.

**Discussion**

Our previous study showed that a 7-day glucose infusion in L-NAME–treated rats increased MAP more than the additive responses to glucose and L-NAME alone. This suggested that nitric oxide synthesis inhibition potentiated the hypertensive response to chronic glucose infusion, and the main finding from the present study is that this effect was shown to be dependent significantly on the sympathetic nervous system. This effect of chronic α1- and β-adrenergic receptor blockade we measured was surprising in light of our previous finding that similar adrenergic blocker treatment did not affect the increase in blood pressure caused by glucose plus insulin infusion in normal rats. That finding was confirmed by the present observation that adrenergic receptor blockade did not alter the blood pressure response to glucose infusion in the vehicle, that is, non–L-NAME–treated, rats, but it is not clear why a role for the sympathetic nervous system was uncovered when glucose was infused in the rats with chronic L-NAME treatment.

**Figure 2.** Change (Δ) in MAP and heart rate during control period (C), 7 days of glucose infusion (G), and during recovery period (R) in the 4 groups of rats. #P<0.05 for L rats vs LB rats.

**Figure 3.** Plasma renin activity in the 4 groups of rats measured during control, glucose infusion, and recovery periods. *P<0.05 compared with control period average for that group; #P<0.05 for L rats vs LB rats; †P<0.05 compared with vehicle group.
We speculate that this may be due to removal of a suppressing influence of nitric oxide on the sympathetic nervous system. Thus, an effect of glucose infusion to stimulate the sympathetic nervous system normally would be counterbalanced by stimulation of nitric oxide and its suppressive effect on sympathetic activity. A stimulatory effect of glucose infusion on nitric oxide could be caused by insulin, because Baron’s laboratory and others have reported that insulin stimulates nitric oxide production sufficiently to have significant cardiovascular actions. In addition, there is good evidence that nitric oxide has a suppressive effect on the sympathetic nervous system, thus, despite data from knockout mouse studies that question the role of nNOS, this suggests that blockade of nitric oxide synthesis with L-NAME would be expected to increase sympathetic activity.

If this is true, however, it is possible that the response to adrenergic receptor blockade we measured in the LB rats was due simply to this effect of L-NAME and was not related to any action of glucose. The top panel of Figure 1, in fact, shows that α and β-adrenergic receptor blockade tended to decrease L-NAME hypertension; however, this was not statistically significant. Moreover, the difference between the two L-NAME groups increased after glucose infusion was begun, and that difference was significant. Thus, adrenergic receptor blockade had a greater effect to decrease blood pressure in L-NAME–treated rats during glucose infusion than during L-NAME treatment alone, and this also is illustrated by the significant difference in the change in blood pressure between the two groups in Figure 2.

Our interpretation, therefore, is that glucose infusion stimulated the sympathetic nervous system, but that effect was evident only when nitric oxide synthesis was blocked. The heart rate data support this interpretation and also help argue that the effect of adrenergic receptor blockade on blood pressure was due to a glucose and nitric oxide (or more accurately, loss of nitric oxide) interaction rather than just due to removal of nitric oxide alone. Figure 1 shows that L-NAME infusion decreased heart rate in both groups, probably caused by the baroreflex, and that even greater reductions occurred in the L-NAME rats with adrenergic receptor blockade (LB rats); that response was attributable to direct chronotropic effects of adrenergic blockade, since blood pressure was lower in that group. Glucose infusion, however, increased heart rate dramatically in the L-NAME rats but not in the L-NAME rats with adrenergic blockade. It is important to note that this large increase in heart rate occurred in the rats with the greatest increase in MAP, suggesting that the heart rate increase was not reflex-mediated but was driven by sympathetic mechanisms. It also is important to note that heart rate returned to baseline during the recovery period after glucose infusion was stopped, providing even stronger evidence that the increase measured in the LB rats was due to glucose and not due solely to loss of nitric oxide per se.

It was surprising, in that regard, that MAP in the L-NAME group did not decrease sooner after stopping glucose infusion. In our previous study, MAP in an L-NAME time-control group, which did not receive glucose infusion, increased gradually during the entire period of study, and MAP in the glucose-infused L-NAME rats decreased rapidly after stopping glucose infusion to equal the blood pressure level in the time-control rats. A comparable decrease in the present study was not reached until day 6 of the recovery period, and the explanation for the delay in the MAP recovery but not the heart rate recovery, is not clear.

It also is not clear why adrenergic receptor blockade only partially attenuated the hypertensive response during glucose infusion but blocked essentially all the heart rate response. As shown in Figure 2, the increase in heart rate during glucose infusion in the L-NAME rats with adrenergic blockade was not different from the increase in the vehicle rats with blockade. Our tests of phenylephrine and propranolol responses suggested we had good adrenergic receptor blockade, and this helps confirm the β-receptor component, thereby suggesting that the modest increase in heart rate in those groups was due to withdrawal of parasympathetic tone. The PRA data in Figure 3 also indicate good β-blockade by showing decreased baseline PRA in vehicle rats and very effective prevention of the increase in PRA during glucose infusion in the L-NAME rats. Our previous study showed that the increase in PRA actually was due almost entirely to L-NAME rather than glucose. This is consistent with our measurements here and previously that PRA tends to decrease during glucose infusion in normal rats; thus, despite being required for the hypertension to develop, angiotensin II does not appear to be the sole mediator of the glucose effect. Moreover, the blockade of the PRA response in the present study, along with the residual hypertension in the L-NAME rats with adrenergic blockade, supports our position that the augmented hypertensive response in the L-NAME group during glucose infusion was due to a glucose and nitric oxide interaction rather than to an effect of L-NAME alone.

It also is possible that thromboxane, which could have a greater effect on blood pressure in the absence of nitric oxide, contributed to the hypertension and may explain at least part of the residual hypertension in the LB rats. We have shown an important role for thromboxane in glucose-induced hypertension, and McNeill’s laboratory has reported similar results in fructose hypertension. In addition, an effect of insulin to increase sensitivity of aldosterone to stimulation by angiotensin II could be considered, but insulin levels were not different between groups, and PRA was significantly lower in the LB rats. We also must consider a contribution from unblocked α- and β-receptors, particularly the former, in light of our heart rate and PRA data that support good blockade of the latter.

Despite the incomplete blockade of the hypertensive response, however, the significant effect of the adrenergic blockers on blood pressure and heart rate provides good evidence that the sympathetic nervous system contributes significantly to the exaggerated hypertension and the increase in heart rate caused by glucose infusion when nitric oxide synthesis is inhibited chronically. Because no such evidence of sympathetic involvement was shown in normal rats, this suggests that the nitric oxide system may be activated during glucose infusion and suppresses sympathetic nervous system.
activity, thereby helping to counteract increases in blood pressure and heart rate. Although we do not know whether nitric oxide actually was stimulated under these conditions, the results suggest that nitric oxide opposes glucose-induced, sympathetic-dependent increases in blood pressure and heart rate. These data and our previous results suggest that the nitric oxide system has a quantitatively greater influence on blood pressure in hyperglycemic and hyperinsulinemic conditions, as occur in type II diabetes, compared with normal conditions. This is consistent with evidence that impaired endothelium-dependent relaxation precedes hypertension in fructose-fed rats and with the hypothesis that such impairment contributes to hypertension in insulin-resistant conditions such as obesity and type II diabetes.

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References

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