Inhibition of Nitric Oxide Synthesis Potentiates Hypertension During Chronic Glucose Infusion in Rats

Christie R. Claxton, Michael W. Brands, Sharyn M. Fitzgerald, Joseph A. Cameron

Abstract—Endothelial dysfunction has been proposed to contribute to impaired blood flow control or hypertension in many conditions characterized by hyperinsulinemia or hyperglycemia. However, most studies have focused on whether endothelial dysfunction is present in the established phases of these various hypertensive states, and there is little known concerning the role of the endothelium in the initial stages. This study tested whether nitric oxide production, before endothelial dysfunction develops, plays an important role in counteracting the hypertensive response to chronic glucose infusion. Glucose was infused (18.6 mg/kg per minute IV) for 7 days in 8 normal rats (G) and in 9 rats with a long-term background intravenous infusion of N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME) at 10 μg/kg per minute (G+L). Mean arterial pressure (MAP), measured 24 hours per day, increased an average of approximately 11 mm Hg in the G rats. L-NAME treatment increased MAP an average of 28±2 mm Hg in the G+L rats, and glucose infusion raised MAP >30 mm Hg above that, averaging 155±8 mm Hg by day 6. In addition, heart rate increased from an average of 389±8 bpm to 441±16 bpm by day 6, whereas there was no significant change in the G rats. Glomerular filtration rate decreased significantly with L-NAME treatment and decreased in both groups by day 3 of glucose infusion, reaching lower levels in the G+L rats. These results show that NO is required to minimize the increase in MAP during glucose infusion and suggest that renal and neural mechanisms may be important in mediating that effect. (Hypertension. 2000;35[part 2]:451-456.)

Key Words: insulin ■ blood pressure ■ glomerular filtration rate ■ endothelium

Endothelial dysfunction, defined in general as an impaired ability of the endothelium to mediate vasodilation, has been proposed to contribute to impaired blood flow control or hypertension in many conditions characterized by hyperinsulinemia or hyperglycemia, such as diabetes, obesity, and insulin-resistant states independent of obesity. One possibility is that factors associated with these disease processes act on the endothelium to progressively impair its vasodilator potential and through that mechanism cause arterial pressure to increase. For example, Katakat et al\textsuperscript{13} reported that impairment of endothelium-dependent relaxation in fructose-hypertensive rats took 2 to 3 weeks to develop and preceded the onset of hypertension. Thus, hypertension, in effect, may be “allowed” to develop as the basal vasodilator influence of the endothelium deteriorates over time, analogous to the increase in arterial pressure caused by blockade of nitric oxide (NO) synthesis under normal conditions.\textsuperscript{14,15}

In addition, it is possible that other mechanisms are responsible for the increase in arterial pressure and that, initially, normal or increased endothelium-mediated vasodilation actually counteracts those actions. Glucose administration and hyperinsulinemia increase arterial pressure in rats, for example, through mechanisms linked to angiotensin II, thromboxane, and endothelin, probably through the control of renal vascular resistance.\textsuperscript{16,18,22–26} In addition, glucose and insulin both have been reported to increase NO production.\textsuperscript{21,27–31} Therefore, glucose-related or insulin-related mechanisms actively exert pressor effects, and the onset and magnitude of any hypertension that develops may depend on the activity of endothelium-derived vasodilators. However, most studies have focused on whether endothelial dysfunction is present in the established phases of these various hypertensive states, and little is known about the role of the endothelium in the initial stages. Thus, the endothelium, before dysfunction develops, may actively oppose the hypertensive influence of factors linked to insulin and glucose.

Our hypothesis, therefore, was that blocking synthesis of the endothelium-derived relaxing factor, NO, during glucose infusion would yield a significantly greater increase in arterial pressure than occurs in rats with a normally functioning NO system. To test this hypothesis, we infused glucose intravenously for 7 days in chronically instrumented rats that received a constant background infusion of either vehicle or N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME).

Methods

All experiments were conducted in male Sprague-Dawley rats (weight ~350 g, Harlan Sprague-Dawley, Madison, Wis), and the
protocols were approved by the Institutional Animal Care and Use Committee at the University of Mississippi Medical Center. Anesthesia was induced with sodium pentobarbital (50 mg/kg IP) and atropine was administered (40 μg IP per rat) to minimize airway secretions. Under aseptic conditions, a laparotomy was performed and a nonocclusive catheter was inserted orthogradely into the abdominal aorta, caudal to the kidneys, through a stab incision in the aortic wall made with the tip of an 18-gauge needle. The catheter was constructed from medical vinyl tubing (BOLAB, size V/4 [20 g]) with one end formed into an S-shape. Into this end was inserted a 5- to 6-mm segment of PE-90 tubing tapered over heat to approximately PE-30 size. Only the PE tip was inserted into the aorta. The insertion point was sealed with cyanoacrylate adhesive and the catheter was exteriorized through the lateral abdominal wall. A femoral vein catheter (BOLAB, size V/4 [20 g]) with no modifications was implanted through a separate incision, and the tip was maneuvered into the vena cava. All incisions were infiltrated with penicillin G and Seroscense at closure, and the catheters were routed subcutaneously to the scapular region and exteriorized through a Dacron-covered stainless steel button sutureted subcutaneously over the scapula.

The rats were allowed to recover from surgery and then were placed in individual metabolic cages in a quiet, air-conditioned room with a 12-hour light cycle. The catheters were passed through a stainless steel spring that was attached to the button, and the opposite end of the spring was connected to a dual-channel hydraulic swivel (Instech). The venous catheter was immediately connected, by means of the hydraulic swivel, to a syringe pump (Harvard Apparatus) that ran continuously throughout the study. All solutions contained antibiotic (25 000 U penicillin G per day per rat) and were infused through a Millipore filter (0.22 μm, Cathivex, Millipore Corp.). The arterial catheter was filled with heparin solution (1000 USP U/mL) and connected, also by means of the swivel, to a pressure transducer (Cobe) mounted on the cage exterior. The amplified pulsatile arterial pressure signals were sent to an analog-to-digital converter and analyzed by computer with customized software. The analog signals were sampled 4 seconds each minute, 24 hours per day.

Sodium intake throughout the experiment was maintained constant at ~2.9 mmol per day by continuous intravenous infusion of 18 mL per day sterile, 0.9% saline, combined with sodium-deficient rat chow (3.04 mL per day sterile, 0.9% saline, combined with sodium-deficient rat chow at 2.9 mmol per day). Sodium intake was maintained at this constant level throughout the experiment. Calcium concentrations were determined with the use of ion-sensitive electrodes (Nova). Results are presented as mean±SEM. Data were analyzed with a repeated-measures ANOVA, and within-group comparisons against control data were made with Dunnett’s test. With a significant between-group F test, specific between-group differences were identified with completely randomized ANOVAs and Fisher least-squares difference testing for each day. Statistical significance was considered to be P<0.05.

Analytical Methods

Glomerular filtration rate (GFR) and effective renal plasma flow were measured by calculating the clearance of 125I-iothalamate and 131I-iodhippuran after 24-hour infusion of the isotopes as described previously.52 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 Accucheck III blood glucose analyzer. Urinary sodium and potassium concentrations were determined with the use of ion-sensitive electrodes (Nova). Results are presented as mean±SEM. Data were analyzed with a repeated-measures ANOVA, and within-group comparisons against control data were made with Dunnett’s test. With a significant between-group F test, specific between-group differences were identified with completely randomized ANOVAs and Fisher least-squares difference testing for each day.51 Statistical significance was considered to be P<0.05.

Results

Mean arterial pressure averaged 91±1, 91±2, and 95±2 mm Hg in the G, L, and G+L rats, respectively, during the precontrol period (time point PC in Figure 1) and increased rapidly in the L and G+L rats with L-NAME infusion, plateauing 31±4 and 28±2 mm Hg higher, respectively, than PC levels by control day 6. Glucose infusion increased mean arterial pressure significantly by day 2 in the G rats, and pressure averaged 102±3 mm Hg, ~11 mm Hg above control levels, for the 7-day experimental period. In the G+L rats, however, mean arterial pressure increased ~13 mm Hg on the first day of glucose infusion and continued to rise, peaking on day 6 of glucose at 60±5 mm Hg above precontrol levels and ~30 mm Hg higher than pressure during L-NAME infusion. After stopping the glucose infusion, pressure returned to control in the G rats; in the G+L rats, arterial pressure decreased to the same level as in the L rats, which had been maintained on L-NAME alone throughout the experiment.

Heart rate was similar in the 3 groups during the precontrol period and decreased significantly in both L-NAME-treated groups during the control period (Figure 2). For the remainder of the experiment, the trend was for heart rate to decrease in the G and L groups (not significant), but in the G+L group there was a significant increase in heart rate during the glucose infusion period that was ~50 bpm above control levels on day 6. During the recovery period, heart rate in the G+L rats decreased to levels not different from the other 2 groups. L-NAME decreased GFR in the L and G+L rats, although the decrease did not reach statistical significance in the L group (Figure 3, Control). During glucose infusion, GFR decreased significantly in the G and G+L rats, similar to previous reports,16,18,22,25,26 and returned toward control levels during the recovery period. Note that GFR decreased significantly in the G+L rats despite starting from a significantly lower baseline. There were no significant changes in GFR in the L group during the experiment. Urinary sodium excretion averaged 2.2±0.3, 2.2±0.2, and 2.5±0.3 mmol/d in the G, L, and G+L rats, respectively, during the precontrol period, and there were no significant changes in sodium excretion in any group during the experiment. There also were no significant
differences in PRA except for increases in both L-NAME–treated groups during the recovery period (Table). Blood glucose and plasma insulin levels increased during the experimental period in the G and G

1

L groups, but the change in insulin in the G+L rats did not reach statistical significance (Table).

Discussion

The main finding in this study was that blockade of NO synthesis with L-NAME greatly exacerbated the increase in arterial pressure induced by chronic glucose infusion. Whereas an ≈11 mm Hg pressure rise occurred in the normal rats infused with glucose, similar to previous studies,18,22,24–26 the increase in the L-NAME rats infused with glucose was ≈30 mm Hg above the L-NAME–induced pressure rise and 60 mm Hg above control levels. This suggests that NO production is increased during glucose infusion and supports our hypothesis that NO plays an important role in blunting the hypertensive response to high glucose.

Impairment in endothelium-mediated vasodilation has been proposed to contribute to the increased arterial pressure in conditions such as obesity6,7 and in rats maintained on a high-fructose diet.8–10,12,13 In insulin-resistant states, some investigators propose that the effect of insulin to induce endothelium-dependent dilation also is impaired.11 Regardless, however, the implication is that NO is required in the normal state to prevent hypertension from developing. This possibility is supported by the observation here and in previous studies14,15 that blockade of NO synthesis in normal animals causes a significant increase in arterial pressure. However, the impairment in endothelial function does not develop instantly, and Katakam et al,13 in fact, reported that impaired vasodilatory function, though preceding the onset of hypertension, took more than 14 days to become significant.

Figure 1. Graph of mean arterial pressure in glucose (G), L-NAME (L), and glucose+L-NAME (G+L) rats during control (C), experimental (E), and recovery (R) periods. *P<0.05 for within-group comparison to the last 4 days of the control period, C3 through C6. #P<0.05 for G+L rats compared with L rats. Note that both L and G+L rats were different from the G rats for each day after L-NAME was started, but symbols were omitted for clarity. fP<0.05 for within-group comparison of control mean arterial pressure in L and G+L rats to their respective precontrol (PC) values.

Figure 2. Graph of heart rate in glucose (G), L-NAME (L), and glucose+L-NAME (G+L) rats during control (C), experimental (E), and recovery (R) periods. *P<0.05 for within-group comparison to the last 4 days of the control period, C3 through C6. #P<0.05 for G+L rats compared with G rats and L rats; single # on G3 indicates P<0.05 for G+L rats compared only with the L rats. L rats were not significantly different from G rats for any day. fP<0.05 for within-group comparison of control heart rate in L and G+L rats to their respective precontrol (PC) values.
Thus endothelial dysfunction would not be expected to mediate the increase in arterial pressure that begins by day 2 of a 7-day glucose infusion.

Our glucose-infusion model uses the same glucose infusion rate that we have used previously to prevent hypoglycemia during chronic insulin infusion,16,18,23–26 and the increases in arterial pressure and plasma insulin are similar.22 The increase in mean arterial pressure is associated with a shift in the renal pressure-natriuresis relationship23 that requires angiotensin II16 and thromboxane18 and also with a decrease in GFR in the middle of the infusion period.16–18,22,25,26 There also is good evidence that insulin-mediated increases in endothelin contribute to the insulin-induced hypertension.19–21 The findings of Koopmans et al34 suggest that insulin alone may not initiate the increase in arterial pressure, so the combined actions of glucose and insulin may be required; nevertheless, mechanisms other than endothelial dysfunction per se appear to mediate the increase in arterial pressure with glucose infusion. Moreover, because endothelial function probably is normal for at least most of the infusion period, it is possible that endothelium-derived vasodilators actually protect against the development of hypertension. The augmented rise in arterial pressure in the glucose-infused rats treated with L-NAME (G L group) in this study support this contention.

The precise mechanism for this greater increase in arterial pressure cannot be discerned from this study but may not be due solely to a direct effect of NO on the vasculature. The marked increase in heart rate in the G + L rats, for instance, is consistent with activation of the sympathetic nervous system. Thus, the glucose infusion may have stimulated NO formation, and part of the action of NO to attenuate the increase in arterial pressure may have been through suppression of the sympathetic nervous system. Blockade of NO synthesis, therefore, would prevent this suppression and enable sympathetic activity to increase. Evidence supporting this possibility is that both glucose and insulin have been reported to increase NO production27–31 and that NO has been reported to suppress sympathetic nervous system activity.35–37 This also is consistent with our previous finding that blockade of α- and β-adrenergic receptors did not prevent the hypertension caused by insulin and glucose infusion.26 This potential role of the sympathetic nervous system, however, does not contradict our hypothesis. Rather, it suggests that NO production by the normal endothelium during glucose infusion could be acting through multiple mechanisms to minimize the increase in arterial pressure.

Another possibility, for example, is interaction with the control of renal vascular resistance through nonsympathetic mechanisms. We have proposed previously16–18 that an increase in tubuloglomerular feedback (TGF) sensitivity may

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Control</th>
<th>Experimental</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, pmol/L</td>
<td>Glucose</td>
<td>220±50</td>
<td>357±46*</td>
<td>312±41</td>
</tr>
<tr>
<td></td>
<td>L-NAME</td>
<td>229±47</td>
<td>224±41</td>
<td>235±49</td>
</tr>
<tr>
<td></td>
<td>G + L</td>
<td>293±54</td>
<td>378±55</td>
<td>275±36</td>
</tr>
<tr>
<td>PRA, ng AI/mL per hour</td>
<td>Glucose</td>
<td>3.5±0.4</td>
<td>3.7±0.6</td>
<td>4.5±0.4</td>
</tr>
<tr>
<td></td>
<td>L-NAME</td>
<td>2.5±0.5</td>
<td>4.4±1.5</td>
<td>6.2±1.1*</td>
</tr>
<tr>
<td></td>
<td>G + L</td>
<td>3.7±0.3</td>
<td>4.4±0.8</td>
<td>5.9±0.7*</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>Glucose</td>
<td>6.4±0.3</td>
<td>8.3±0.3*</td>
<td>7.0±0.2</td>
</tr>
<tr>
<td></td>
<td>L-NAME</td>
<td>6.8±0.2</td>
<td>7.0±0.2</td>
<td>6.5±0.2</td>
</tr>
<tr>
<td></td>
<td>G + L</td>
<td>6.6±0.2</td>
<td>8.5±0.4*</td>
<td>7.0±0.2</td>
</tr>
</tbody>
</table>

*P<0.05 compared with control.
Contribute significantly to the decrease in GFR observed in this model, and there is good evidence that NO blunts TGF sensitivity.\textsuperscript{38,39} Thus, although the percent decrease in GFR during glucose infusion was similar in the G and G+L rats, the decrease in the G+L rats was from a significantly lower baseline. That is consistent, at least, with the possibility that withdrawal of the blunting effect of NO on TGF sensitivity by L-NAME treatment could have contributed to the lower GFR in that group during glucose infusion and thereby provided a potential mechanism for the greater increase in arterial pressure.

The role of angiotensin II is not clear. PRA normally decreases in this experimental model,\textsuperscript{16,18,23,25,26} although no significant change was measured in the G rats in this study. However, the hypertension is angiotensin II dependent,\textsuperscript{16} possibly related to its powerful effect on TGF sensitivity.\textsuperscript{40,41} In both L-NAME–treated groups in this study, PRA began to increase in the experimental period and was significantly greater than control by the recovery period. Because we have shown that preventing angiotensin II from decreasing during insulin and glucose infusion exacerbates the increase in arterial pressure,\textsuperscript{17} this suggests that the increase in angiotensin II associated with L-NAME treatment in this study could have contributed to the greater increase in MAP in the G+L versus G rats. However, additional studies will be required to determine the role of the renin-angiotensin system in mediating the augmented hypertensive response to glucose infusion with NO synthesis blocked.

It also is not clear whether NO synthesis actually increased during glucose infusion or, if so, what the mechanism might be. There is evidence from 2-kidney\textsuperscript{42} and 1-kidney\textsuperscript{43,44} Goldblatt hypertension that increased arterial pressure can increase NO synthesis, and stimulation of the sympathetic nervous system also has been shown to induce NO release.\textsuperscript{45} The potential role of negative feedback with the sympathetic system is intriguing, given our data suggesting that NO may have exerted a suppressing influence on sympathetic activity. In addition, however, both glucose and insulin can increase NO production\textsuperscript{21,27–31}; in fact, Cardillo et al\textsuperscript{21} showed recently that insulin simultaneously increased endothelin and NO release, further strengthening our hypothesis that NO production from a healthy endothelium is important to counteract insulin-linked or glucose-linked hypertensive stimuli. Thus it is likely that NO synthesis was increased during glucose-induced hypertension, but additional studies will be needed to quantify the roles of pressure-dependent and glucose-dependent mechanisms.

These results indicate, therefore, that during the initial stages of glucose loading and hyperinsulinemia in rats, before development of endothelial dysfunction, NO synthesis is important to attenuate the increase in arterial pressure. The mechanism for this effect is not known but could include actions to decrease the activity or influence of the sympathetic nervous system, TGF feedback, endothelin, or the renin-angiotensin system.

Acknowledgments
This research was supported by Heart, Lung, and Blood Institute grants HL-56259 and HL-51971, and HL-07635 in part, and conducted during the tenure of an Established Investigator Grant from the American Heart Association and Genentech. Dr Fitzgerald is the recipient of a postdoctoral fellowship award from the Mississippi Affiliate of the American Heart Association Southern Research Consortium. We thank Allison Hailman and Rico McGowan for technical assistance.

References


Inhibition of Nitric Oxide Synthesis Potentiates Hypertension During Chronic Glucose Infusion in Rats

Christie R. Claxton, Michael W. Brands, Sharyn M. Fitzgerald and Joseph A. Cameron

_Hypertension_. 2000;35:451-456
doi: 10.1161/01.HYP.35.1.451

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2000 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/35/1/451

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Hypertension_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Hypertension_ is online at:
http://hyper.ahajournals.org//subscriptions/