Subpressor Dose of L-NAME Unmasks Hypertensive Effect of Chronic Hyperinsulinemia

Michael Bursztyn, Judith Mekler, Edna Peleg, Jacques Bernheim

Abstract—We previously found that chronic exogenous hyperinsulinemia without sugar supplementation does not elevate blood pressure. This may be partially explained by the ability of insulin to release nitric oxide and cause vasodilatation. To test this hypothesis, we studied 4 groups of rats: 9 rats (body weight, 213±14 g) treated with a gradual increase of a sustained-release subcutaneous insulin pellet; 9 rats (body weight, 213±9 g) treated with N^G^-nitro-L-arginine methyl ester (L-NAME) in drinking water 50 mg/L; 19 rats (body weight, 217±11 g) treated with the combination of L-NAME and insulin; and 9 control rats (body weight, 218±11 g). Blood pressure was followed weekly for 6 weeks, and then rats were studied in metabolic cages. Weight gain was not different during the 6 weeks. Renal function did not differ between the 4 groups, but 24-hour urinary nitrite/nitrate excretion was lower (P<0.02) in L-NAME–treated and higher in insulin-treated rats. Plasma insulin doubled (P<0.002) in the insulin-treated rats, but there was no hypoglycemia and, by week 6, fructosamine levels were 2.1±0.2, 2.1±0.2, 2.3±0.1, and 2.3±0.2 mmol/L in control rats and rats treated with L-NAME, insulin, and L-NAME plus insulin, respectively. Systolic blood pressure, which did not differ at baseline, at week 3 was 122±17, 118±17, and 118±24 mm Hg in the control, L-NAME, and insulin groups and 136±14 mm Hg (P<0.03) in the combination group. At week 6, systolic blood pressure was 128±14, 127±15, and 118±13 mm Hg in the control, L-NAME, and insulin groups, respectively, and 150±14 mm Hg (P<0.0005) in the combination group. In a subsequent experiment, L-arginine 2 g/L abrogated the effects of L-NAME and insulin combination. In conclusion, chronic exogenous hyperinsulinemia does not affect blood pressure but may cause hypertension when endothelial function is compromised. (Hypertension. 2000;36:872-877.)

Key Words: insulin • nitric oxide • sodium • nitrates • nitrites

Hyperinsulinemia was found to be common in essential hypertension even without obesity and is the foundation for the concept of the metabolic syndrome X. However, the manner in which insulin elevates blood pressure is unclear. Sympathetic activation,1,2 sodium retention,3 and vascular hypertrophy have been suggested4 but never proven in a chronic model of hyperinsulinemia. Indeed, because of the vasodilatory effects of insulin,5 it has been suggested the vasodilation may mask a hyperinsulinemia. Indeed, because of the vasodilatory effects of insulin,5 it has been suggested the vasodilation may mask a hyperinsulinemia. Indeed, because of the vasodilatory effects of insulin,5 it has been suggested the vasodilation may mask a hyperinsulinemia. Indeed, because of the vasodilatory effects of insulin,5 it has been suggested the vasodilation may mask a hyperinsulinemia. Indeed, because of the vasodilatory effects of insulin,5 it has been suggested the vasodilation may mask a hyperinsulinemia. Indeed, because of the vasodilatory effects of insulin,5 it has been suggested the vasodilation may mask a hyperinsulinemia. Indeed, because of the vasodilatory effects of insulin,5 it has been suggested the vasodilation may mask a hyperinsulinemia. Indeed, because of the vasodilatory effects of insulin,5 it has been suggested the vasodilation may mask a hyperinsulinemia. Indeed, because of the vasodilatory effects of insulin,5 it has been suggested the vasodilation may mask a hyperinsulinemia. Indeed, because of the vasodilatory effects of insulin,5 it has been suggested the vasodilation may mask a hyperinsulinemia. Indeed, because of the vasodilatory effects of insulin,5 it has been suggested the vasodilation may mask a hyperinsulinemia. Indeed, because of the vasodilatory effects of insulin,5 it has been suggested the vasodilation may mask a hyperinsulinemia. Indeed, because of the vasodilatory effects of insulin,5 it has been suggested the vasodilation may mask a hyperinsulinemia. Indeed, because of the vasodilatory effects of insulin,5 it has been suggested the vasodilation may mask a hyperinsulinemia. Indeed, because of the vasodilatory effects of insulin,5 it has been suggested the vasodilation may mask a hyperinsulinemia. Indeed, because of the vasodilatory effects of insulin,5 it has been suggested the vasodilation may mask a hyperinsulinemia. Indeed, because of the vasodilatory effects of insulin,5 it has been suggested the vasodilation may mask a hyperinsulinemia. Indeed, because of the vasodilatory effects of insulin,5 it has been suggested the vasodilation may mask a hyperinsulinemia. Indeed, because of the vasodilatory effects of insulin,5 it has been suggested the vasodilation may mask a hyperinsulinemia. Indeed, because of the vasodilatory effects of insulin,5 it has been suggested the vasodilation may mask a hyperinsulinemia. Indeed, because of the vasodilatory effects of insulin,5 it has been suggested the vasodilation may mask a hyperinsulinemia. Indeed, because of the vasodilatory effects of insulin,5 it has been suggested the vasodilation may mask a hyperinsulinemia. Indeed, because of the vasodilatory effects of insulin,5 it has been suggested the vasodilation may mask a hyperinsulinemia. Indeed, because of the vasodilatory effects of insulin,5 it has been suggested the vasodilation may mask a hyperinsulinemia.

There have been several attempts to produce hypertension in rats by way of chronic exogenous hyperinsulinemia.6–10 In most of these studies, increase in carbohydrate consumption (intravenous glucose or oral sucrose) was combined with insulin to produce chronic exogenous hyperinsulinemia in normal rats, without sugar supplementation and without hypoglycemia, that did not affect blood pressure.10 However, when the nitric oxide (NO) system may be defective, such as in Sabra hypertension-prone rats,11 spontaneously hypertensive rats,12 and Dahl salt-sensitive rats,13 then hyperinsulinemia without sugar supplementation may cause hypertension.7,12,14 When the NO system is maximally activated, as it may be in pregnancy, then hyperinsulinemia is also associated with hypertension.15,16 We therefore attempted to combine chronic exogenous hyperinsulinemia with a subpressor dose of the NO inhibitor N^G^-nitro-L-arginine methyl ester (L-NAME) with the hypothesis that a subclinical defect in the ability for NO generation may bring about the prohypertensive effect of insulin.

Methods

Rats

Regular male Sprague-Dawley rats weighing ~200 g were obtained from Anilab (Rehovot, Israel). Rats were housed in regular cages (4 rats to a cage) and maintained on standard rat chow (Kofflok) containing 56% grain-derived carbohydrate, 20% protein, 13% moisture, 5.3% cellulose, 3% fat, 0.8% calcium, 0.6% phosphorus, and 0.3% NaCl, with free access to tap water. They were maintained on a 12-hour light/dark cycle. All animals were handled and housed according to the guidelines and manual of the Committee on the Care of Laboratory Animals of the Hebrew University–Hadassah Medical School.

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Blood Pressure Determination
Rats were weighed and had their systolic blood pressure (SBP) measured weekly (prewarmed) by the tail-cuff method (IITC, Life Sciences).

Procedures
After habituation to blood pressure recording, the rats were randomly allocated to 1 of 4 groups. Insulin-treated rats (body weight, 213±14 g; n=9) received a 2-mm segment of a 7-mm sustained-release insulin implant (Linplant, Linshin Canada Inc) designed to deliver ~2 U/d for >40 days by means of a 12-gauge hypodermic needle under short-term ether anesthesia. Control rats (body weight, 218±11 g; n=9) underwent sham implantation under the same conditions. The L-NAME group (body weight, 213±9 g; n=9) received 50 mg/L in their drinking water. The combination group (body weight, 217±11 g; n=19) received both insulin and L-NAME. Two weeks after the first implantation, insulin-treated rats were implanted with a 3.5-mm sustained-release insulin implant. This schedule was determined after extensive preliminary testing and was

Figure 1. Insulin (top), glucose (middle), and fructosamine (bottom) levels after 3 and 6 weeks of exogenous insulin treatment. *P<0.03 vs control and L-NAME–treated groups by 1-way ANOVA.
previously found not to produce hypoglycemia in normal rats. Tail blood was obtained at weeks 3 and 6 for determination of plasma glucose by an ultraviolet enzymatic test with glucose dehydrogenase (Glucose GDH; Hoffman-LaRoche) and urinary NO urinary metabolites and norepinephrine excretion.

In a subsequent experiment we added l-arginine 2 g/L to the drinking water of 28 rats to test whether it could prevent the effect of the L-NAME/insulin combination on blood pressure. Twenty-three rats received L-NAME and insulin, as in the previous experiment, and were divided into 3 groups: L-NAME+insulin combination (n=4); L-NAME+insulin+l-arginine (from day 1) (n=12); and L-NAME+insulin+l-arginine (from week 4) (n=9). Four additional rats received l-arginine alone throughout. All other procedures were as in the previous experiment except for the additional measurement of heart rate and except for observation of rats for 8 weeks when the metabolic cages studies were performed.

Statistical Analysis
Data are presented as mean±SD except for the figures in which SE are shown. Statistical analysis was performed on Crunch software (version 4, 1991). ANOVA was used for between-group comparison with Newman-Keuls post hoc analysis. P<0.05 was considered significant.

Results
During the 6 weeks of the study, there was no difference in body weight between the groups (data not shown). As of week 3, insulin levels were more than doubled (P<0.002) in the insulin and insulin/L-NAME groups (Figure 1, top panel). However, this doubling of insulin plasma levels did not cause hypoglycemia. There was a decrease in glucose levels in week 3 (P<0.002) in the insulin-treated groups (Figure 1, middle panel), which did not persist to week 6. Moreover, the equivalence of fructosamine levels (a measure of integrated glycemia levels and protein nonenzymatic glication) in all groups after both 3 and 6 weeks of insulin treatment (Figure 1, bottom panel) demonstrates absence of persistently low glucose levels in the insulin-treated rats.

SBP throughout the experiment is shown in Figure 2. Neither insulin nor L-NAME alone affected SBP in the rats. However, by week 6 SBP was higher (P<0.0005) in the insulin/L-NAME combination group than the control group by 23 mm Hg, than the L-NAME group by 23 mm Hg, and than the insulin group by 32 mm Hg. The increase of SBP is evident and significant (P<0.02) from week 3 onward. Results of the metabolic cages study are shown in Table 1. There were no differences in water intake or urine volume, urinary Na+ or K+ excretion, or fractional Na+ excretion. However, norepinephrine excretion was lower than control in the L-NAME group (P<0.04). The excretion of nitrites/nitrates was lower in the L-NAME group than in the control (P<0.05) and insulin-treated groups (P=0.01). Plasma nitrates by week 3 were 3.2±1.7, 1.7±4.0, 2.8±1.7, and 2.0±2.1 μmol/L in the control, L-NAME, insulin, and insulin+L-NAME groups, respectively; by week 6 they were 3.2±1.7, 1.7±0.8, 3.8±2.7, and 2.4±1.7 μmol/L, respectively.

The SBP measurements of the second experiment are shown in Figure 3. The L-NAME+insulin combination group had a significantly elevated SBP at 165±15 mm Hg (P<0.05 versus baseline). This blood pressure elevation was not evident in the arginine and L-NAME+insulin+l-arginine groups, whose SBP by the end of the experiment leveled at 116±24 and 141±10 mm Hg, respectively, as did the L-NAME+insulin+l-arginine group at 132±13 mm Hg. However, in this group SBP was elevated (until L-arginine treatment) to 160±10 mm Hg (P<0.003 versus baseline). Overall, 2-way ANOVA found that SBP in the L-NAME+ insulin combination group rose to significantly higher levels than all the l-arginine–treated groups (F2,73=8.02, P=0.0007). Weight did not differ between the groups in the second experiment (data not shown). Heart rate did not differ between the various L-NAME+insulin groups (Figure 4), but the l-arginine

### Table 1. Twenty-Four Hour Metabolic Studies

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Drinking Volume, mL</th>
<th>Urine Volume, mL</th>
<th>Urinary Na+ Excretion, mmol/24 h</th>
<th>Urinary K+ Excretion, mmol/24 h</th>
<th>Creatinine Clearance, mL/min</th>
<th>Fractional Na+ Excretion, %</th>
<th>Urinary Nitrite/Nitrate Excretion, μmol/24 h</th>
<th>Urinary Norepinephrine Excretion, μmol/24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9</td>
<td>39.1±6.1</td>
<td>12.2±5.6</td>
<td>1.5±0.1</td>
<td>0.36±0.14</td>
<td>1.6±0.1</td>
<td>0.004±0.0001</td>
<td>21.6±2.5</td>
<td>8.8±1.3</td>
</tr>
<tr>
<td>L-NAME</td>
<td>8</td>
<td>38.7±7.4</td>
<td>11.4±3.0</td>
<td>1.6±0.1</td>
<td>0.34±0.09</td>
<td>1.8±0.1</td>
<td>0.004±0.0001</td>
<td>13.9±1.2‡</td>
<td>5.8±1.8‡</td>
</tr>
<tr>
<td>Insulin</td>
<td>9</td>
<td>33.2±11.2</td>
<td>12.8±3.6</td>
<td>1.6±0.2</td>
<td>0.38±0.10</td>
<td>1.9±0.3</td>
<td>0.004±0.001</td>
<td>24.3±2.9</td>
<td>8.1±1.2</td>
</tr>
<tr>
<td>Insulin/L-NAME</td>
<td>19</td>
<td>36.4±14.9</td>
<td>11.5±6.1</td>
<td>1.5±0.1</td>
<td>0.34±0.10</td>
<td>1.8±0.1</td>
<td>0.004±0.001</td>
<td>16.6±1.7</td>
<td>6.6±0.9</td>
</tr>
</tbody>
</table>

*Values for norepinephrine excretion were control 4, L-NAME 4, insulin 5, and L-NAME+insulin 11.
‡P<0.05 vs control.
‡P=0.01 vs insulin.
group had a higher heart rate, which was present at the outset. After we controlled this baseline difference by entering baseline heart rate as a covariate, 2-way ANCOVA still found the heart rate of this group to be faster ($F_{27,3} = 3.58, P < 0.03$).

Glucose and nitrate levels at 3 and 6 weeks are shown in Table 2. There were no significant differences between the groups’ urine volume, water drinking volume, creatinine clearance, urinary nitrates/nitrites, and norepinephrine values, as shown in Table 3. There was no difference in renal function or norepinephrine excretion between the groups. However, nitrite/nitrate excretion was different between the groups by ANOVA ($F_{27,3} = 5.9, P = 0.0045$). The excretion was significantly higher in the 2 groups that drank $L$-arginine throughout the experiment.

**Discussion**

The main and new finding in this study is the clear and substantial hypertensive effect of insulin when combined with a subpressor dose of the NOS inhibitor L-NAME. The hypertension caused by the insulin/L-NAME combination was not associated with renal dysfunction or salt-handling abnormality, since creatinine clearance, urinary Na$^+$ and K$^+$ excretion, and fractional Na$^+$ excretion did not differ between the groups. Originally, we hypothesized that increase in NO production by insulin may account for the lack of effect of chronic exogenous hyperinsulinemia on blood pressure in our laboratory.$^{19}$ We found here that a subpressor dose of L-NAME significantly reduces excretion of nitrites/nitrates without affecting plasma levels and that this reduced excretion is reversible with $L$-arginine. This indicates that the low L-NAME dose used here had an inhibitory effect on NOS. Why this was not evident in plasma nitrites is not clear, but levels tended to be lower in the L-NAME–treated rats, although not significantly. Nevertheless, because of the complexity of the NO system, point determinations may have overlooked changes. Neither urinary norepinephrine levels nor heart rate indicates that the putative role of the sympathetic nervous system is not operative.$^1$ The lower urinary norepinephrine excretion in the L-NAME–treated group may be baroreflex mediated as a result of the new steady state after NOS inhibition. There is a plethora of conflicting experimental results for the role of neuronal NOS in NOS inhibitor–induced hypertension.$^{20}$ Such differences may be explained in part by anesthesia, short-term versus long-term experiments, specificity of various NOS inhibitors, and species differences. Our results do not point to a specific role of the sympathetic nervous system, although the reduction in norepinephrine with a subhypertensive L-NAME dose, as well as the higher heart rate with $L$-arginine, confirms some interplay between NO, insulin, and sympathetic activity. However, it is possible that both sympathetic nervous system actions of insulin$^{1,2}$ and those of L-NAME$^{20}$ interacted to produce hypertension even when neither of them alone affected SBP. Increased sympathetic nervous system activity may go undetected by urinary norepinephrine or heart rate. Previously, unlike our earlier findings,$^{10,12,19}$ hyperinsulinemia accompanied by glucose infusion reportedly caused hypertension.$^6$ Recently, a report

**TABLE 2. Plasma Glucose and Nitrates in Rats Treated With L-NAME/Insulin and/or $L$-Arginine**

<table>
<thead>
<tr>
<th>Group</th>
<th>Week 3</th>
<th>Week 6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose, mmol/L</td>
<td>Nitrates, $\mu$mol/L</td>
</tr>
<tr>
<td>Insulin/L-NAME</td>
<td>n=4 4.6±0.9</td>
<td>1.2±1.8</td>
</tr>
<tr>
<td>$L$-Arginine</td>
<td>n=5 5.4±0.4</td>
<td>4.3±5.8</td>
</tr>
<tr>
<td>Insulin/L-NAME+$L$-arginine</td>
<td>n=9 4.6±0.7</td>
<td>5.7±7.1</td>
</tr>
<tr>
<td>Insulin/L-NAME+late $L$-arginine</td>
<td>n=7 4.8±0.6</td>
<td>2.8±3.4</td>
</tr>
</tbody>
</table>
from the same laboratory found that L-NAME–induced hypertension may be exacerbated by hyperinsulinemia produced by glucose infusions.21 These observations indicate that some of the earlier findings of Brands et al6 may have been the result of the effect of glucose rather than insulin infusion, as predicted by the effects of sugar supplementation alone on SBP.22 However, they do point to an interaction of insulin with the NO system. Our finding that the hypertensive effect of combining insulin with a subpressor dose of L-NAME is both preventable with l-arginine and treatable after hypertension has been established supports the crucial role of the defective NO system to allow hypertension during chronic hyperinsulinemia, especially since l-arginine returned (at least in the prevention group) nitrite/nitrate excretion to control levels.

Our method of chronic hyperinsulinemia is devoid of the confounding effect of carbohydrate supplementation. We have taken great care to gradually increase the dose of insulin to prevent hypoglycemia.10 Although glucose levels were lower by week 3 in the first experiment, there was no obvious hypoglycemia, and during the second experiment glucose was not affected (Table 2). Moreover, fructosamine levels, a measure of integrated glycemia,11,12,14–16 and could be explained, in part, by insulin blunting its own effect by activating the NO system. Our finding that the hypertensive effect of combining insulin with a subpressor dose of L-NAME is both preventable with L-arginine and treatable after hypertension has been established supports the crucial role of the defective NO system to allow hypertension during chronic hyperinsulinemia, especially since L-arginine returned (at least in the prevention group) nitrite/nitrate excretion to control levels.

TABLE 3. Drinking Volume, Urine Volume, Creatinine Clearance, Nitrites/Nitrates, and Norepinephrine Excretion in Rats Treated With L-NAME and/or L-Arginine

<table>
<thead>
<tr>
<th>Group</th>
<th>Drinking Volume, mL</th>
<th>Urine Volume, mL</th>
<th>Creatinine Clearance, mL/min</th>
<th>Urinary Nitrites/Nitrates, nmol/24 h</th>
<th>Urinary Norepinephrine, nmol/24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin/L-NAME</td>
<td>n=4</td>
<td>33.0±9.5</td>
<td>11.5±6.4</td>
<td>1.5±0.1</td>
<td>14.5±6.5</td>
</tr>
<tr>
<td>L-arginine</td>
<td>n=5</td>
<td>35.8±10.1</td>
<td>13.3±3.1</td>
<td>1.6±0.2</td>
<td>23.7±3.7*</td>
</tr>
<tr>
<td>Insulin/L-NAME+l-arginine</td>
<td>n=9</td>
<td>33.0±7.3</td>
<td>13.8±3.2</td>
<td>1.4±0.3</td>
<td>19.2±3.8</td>
</tr>
<tr>
<td>Insulin/L-NAME+Late l-arginine</td>
<td>n=7</td>
<td>32.7±13.4</td>
<td>10.5±4.2</td>
<td>1.5±0.2</td>
<td>13.2±5.2</td>
</tr>
</tbody>
</table>

*P<0.005 vs insulin/L-NAME and insulin/L-NAME+l-arginine.

References

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