Hypertension in L-NAME-treated diabetic rats depends on an intact sympathetic nervous system

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Fitzgerald, Sharyn M., and Michael W. Brands. Hypertension in L-NAME-treated diabetic rats depends on an intact sympathetic nervous system. Am J Physiol Regulatory Integrative Comp Physiol 282: R1070–R1076, 2002.—We demonstrated previously that induction of diabetes in rats that were treated chronically with the nitric oxide synthase inhibitor Nω-nitro-L-arginine methyl ester (L-NAME) causes a severe, progressive increase in mean arterial pressure. This study tested the role of the sympathetic nervous system in that response. Rats were Instrumented with chronic artery and vein catheters and assigned randomly to four diabetic groups pretreated with vehicle (D), L-NAME (D + L), the α1- and β-adrenergic receptor antagonists terazosin and propranolol (D + B), or L-NAME, terazosin, and propranolol (D + LB). After baseline measurements were taken, rats were pretreated; 6 days later, streptozotocin was administered and 3 wk of diabetes ensued. D + L rats had a marked, progressive increase in arterial pressure that by day 20 was ~60 mmHg greater than in D rats. The pressor response to L-NAME was significantly attenuated in diabetic rats cotreated with adrenergic blockers. During week 1 of diabetes, plasma renin activity (PRA) increased and then returned to control levels in D rats. PRA increased progressively in D + L rats, and chronic adrenergic receptor blockade restored the biphasic renin response in D + LB rats. These results suggest that the sympathetic nervous system may be involved in the hypertensive response to onset of diabetes in L-NAME-treated rats, possibly through control of renin secretion.

nitric oxide; mean arterial pressure; glucose; angiotensin II; glomerular filtration rate; Nω-nitro-L-arginine methyl ester

DIABETES GENERALLY IS ASSOCIATED with an impairment in endothelial function (10, 34, 35, 37, 43). The long-term consequences of this impairment have not been well established, but it may play an important role in the accelerated atherosclerosis and increased prevalence of cardiovascular disease that is observed in diabetic populations (45a). Also unclear is what role the endothelium and its vasoactive hormones play in circulatory homeostasis early in diabetes before significant impairment develops (4, 20, 34, 44). We demonstrated recently (15) that the induction of diabetes in the absence of a functional nitric oxide (NO) system causes a severe, progressive increase in mean arterial pressure (MAP). This finding suggests that there may be increased dependence on NO in the early stages of diabetes such that NO is essential to prevent hypertension from developing. This is consistent with reports (10, 31) that NO production is increased during the early stages of diabetes.

The mechanism for this potentiating interaction on blood pressure between induction of diabetes and chronic NO synthesis inhibition is not known, but a role for the renin-angiotensin system was implicated by the finding of increased plasma renin activity (PRA) in the rats in our study (15). Increased PRA also is consistent with increased sympathetic nervous system activity, and the progressive increase in heart rate that we measured over the second and third weeks of diabetes in the hypertensive rats (15) further hinted of a role for the sympathetic nervous system. Moreover, because there is evidence that NO may have a suppressing influence on sympathetic activity (48), it is possible that withdrawal of that influence could play a role in the accelerated arterial pressure increase in Nω-nitro-L-arginine methyl ester (L-NAME)-treated diabetic rats (15). This study tested that hypothesis by repeating the experiment in rats with chronic blockade of α- and β-adrenergic receptors.

METHODS

The experiments were conducted on 33 male Sprague-Dawley rats (body wt 329 ± 3 g; Harlan Sprague Dawley, Madison, WI). Protocols were approved by the Institutional Animal Care and Use Committee. Anesthesia was induced with pentobarbital sodium (50 mg/kg ip), and atropine (40 μg/rat ip) was administered to ensure an unobstructed airway. Body temperature was maintained at ~37°C using a servo-controlled heating pad. Under aseptic conditions, an artery and a vein catheter were implanted as described previously (4, 6, 7, 15). All incisions were infiltrated with penicillin G procaine (300,000 U/ml) and Sensocaine at closure, and both catheters were routed subcutaneously to the scapular region and exteriorized.

After the rats had recovered from anesthesia, they were placed in individual metabolic cages. The catheters were connected to dual-channel hydraulic swivels (Instech, Ply-
mouth Meeting, PA) mounted above the cages. The venous catheter was connected via the hydraulic swivel to a syringe pump (Harvard Apparatus, Millis, MA) that ran continuously throughout the study. Sodium intake throughout the experiment was controlled by continuous intravenous infusion of 25 ml/day of sterile 0.9% saline combined with feeding of sodium-deficient rat chow (0.006 mmol sodium/g; Teklad, Madison, WI). The arterial catheter was filled with heparin solution (1,000 USP U/ml) and connected (also via the hydraulic swivel) to a pressure transducer (Cobe, Lakewood, CO) mounted on the cage at the level of the rat. Pulsatile arterial pressure signals were amplified, sent to an analog-to-digital converter, and analyzed by computer using customized software. The analog signal was sampled at 4 s/min for 24 h/day.

Experimental protocol. The rats were divided randomly into four diabetic groups pretreated with vehicle (D, n = 9); the NO synthase inhibitor L-NAME (D + L, n = 10); the α1-receptor antagonist terazosin and the β-adrenergic receptor antagonist propranolol (D + B, n = 6); and L-NAME, terazosin, and propranolol (D + LB, n = 8). (Owing to rat dropout from arterial catheter failure before the end of the protocol, n values are unequal.) After 5 days of baseline measurements, the appropriate drug [L-NAME (10 μg·kg⁻¹·min⁻¹ iv), terazosin (4.17 μg·kg⁻¹·min⁻¹ iv), propranolol (6.94 μg·kg⁻¹·min⁻¹ iv), or vehicle] was added to the infused. This continuous infusion was maintained throughout the remainder of the study. Six days after the administration of specific drugs or vehicle, streptozotocin (50 mg/kg iv) was administered to all rats and a 3-wk diabetic period ensued. To maintain moderate hyperglycemia, insulin (20–25 mmol/l) was added to the daily infusate as needed based on daily blood glucose measurements that were performed during the normal catheter-flushing procedures as previously described (4, 15). On day 4 of the control period and once per week during the diabetic period, 1.4 ml of arterial blood was collected from the arterial catheter after a 4-h fast and was placed in chilled sodium EDTA tubes for measurement of glomerular filtration rate (GFR) and PRA. Samples were replaced with equal volumes of 0.9% saline. 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.

Analytical methods. GFR was measured using a 4-h fasting plasma sample after a 24-h intravenous infusion of [¹²⁵I]iothalamate (0.015 C·kg⁻¹·min⁻¹; Glofil). Because steady state is achieved during the 24-h infusion, the isotope infusion rate was substituted for urinary isotope excretion rate to calculate clearance. Urinary sodium and potassium concentrations were determined using ion-sensitive electrodes (NOVA, Waltham, MA). PRA was measured by radioimmunoassay of ANG I.

Daily hemodynamic data were analyzed by ANOVA with repeated measures and Dunnett’s t-test. Supplemental between-group comparisons for individual days were made using a completely randomized ANOVA with t-tests for differences among several means. A value of P < 0.05 was considered statistically significant, and data are presented as means ± SE.

RESULTS

MAP averaged 96 ± 2, 92 ± 3, 89 ± 1, and 91 ± 2 mmHg in the D, D+B, D+L, and D+LB groups, respectively, during the baseline precontrol period (Fig. 1). By day 6 of the control period, adrenergic blockade had lowered baseline MAP by 10 ± 2 mmHg in the D+B rats, whereas L-NAME caused an increase in MAP that plateaued at ~26 mmHg above baseline levels in the D+L rats. The pressor response to L-NAME treatment was abolished in the the D+LB rats, which were also subjected to adrenergic blockade (P < 0.001; Fig. 1). After the induction of diabetes, there was a marked, progressive increase in MAP in the D+L rats that was ~60 mmHg greater than in the D rats by day 20 of diabetes (P < 0.001; Fig. 2), which is similar to our previous finding (15). By contrast, in diabetic rats treated with both L-NAME and adrenergic receptor blockers (D+LB), MAP was only 10 mmHg greater than in the D rats and 20 mmHg greater than in the D+B rats by day 20 of diabetes (P > 0.05; Fig. 2). Thus the increase in arterial pressure during diabetes in L-NAME-treated rats (40 mmHg) was attenuated in diabetic rats in which both NO synthesis and adrenergic receptor.
duced a marked increase in urinary sodium excretion respectively. However, the induction of diabetes period. There were no signif- cates that the rise in arterial pressure in the L-NAME- sodium excretion between the four groups, which indi- 

Heart rates were not different between groups dur- during the precontrol period. L-NAME treatment de- creased heart rate by 37 ± 7 beats/min by day 6 of the control period, whereas adrenergic blockade decreased heart rate by 57 ± 2 beats/min; combination therapy decreased heart rate by 58 ± 4 beats/min (P < 0.001; see Fig. 1). Induction of diabetes decreased heart rate in all four groups by ~75 beats/min over the first week. The bradycardia was maintained in the D and D+B rats and averaged 93 ± 4 and 83 ± 2 beats/min, respectively, below control levels by day 20 of the diabetic period (P < 0.001). In contrast, the bradycardic response to diabetes waned in the L-NAME-treated (D+L) rats as we showed previously (15) with heart rate averaging 46 ± 6 beats/min below the control level by day 20 of diabetes. This response was not altered by chronic adrenergic blockade (in D+LB rats). Thus blockade of NO synthesis attenuated the bradycardia associated with induction of diabetes, and this effect of L-NAME was not mediated by increased adrenergic activity.

During the control period, urinary sodium excretion was not different between the four groups and averaged 2.8 ± 0.3, 3.4 ± 0.1, 3.3 ± 0.2, and 3.6 ± 0.2 mmol/day for the D, D+B, D+L, and D+LB rats, respectively. However, the induction of diabetes produced a marked increase in urinary sodium excretion in all four groups of diabetic rats (P < 0.001; see Fig. 1) that was maintained throughout the 3-wk diabetic period. There were no significant differences in urinary sodium excretion between the four groups, which indicates that the rise in arterial pressure in the L-NAME-treated diabetic rats was not due to volume retention.

During the control period, GFR was not different between the four groups and averaged 3.4 ± 0.1, 3.2 ± 0.1, 3.1 ± 0.1, and 3.2 ± 0.1 ml/min in the D, D+B, D+L, and D+LB rats, respectively (Fig. 3). With the induction of diabetes, GFR increased in all four groups of rats (P < 0.001; Fig. 3). However, this increase in GFR was attenuated in both groups of L-NAME-treated diabetic rats (between-group P = 0.042; D+L and D+LB groups were significantly different from D group on weeks 2 and 3 with P < 0.05). This is consistent with our previous findings (15), and suggests that NO may play a role in mediating the hyperfiltration that is observed in diabetes.

L-NAME treatment significantly decreased baseline PRA, which averaged 2.74 ± 0.21 and 2.02 ± 0.18 ng ANG I·ml⁻¹·h⁻¹ in the D+L and D+LB rats, respectively, compared with 4.06 ± 0.13 and 3.66 ± 0.25 ng ANG I·ml⁻¹·h⁻¹ in the D and D+B rats, respectively (P < 0.001; Fig. 4). PRA increased during the first week of diabetes in the normal diabetic rat (D) as we showed previously (5, 15). Furthermore, PRA returned to control levels during the second and third weeks, which also is consistent with our previous report (15). L-NAME, on the other hand, decreased baseline PRA levels and caused PRA to increase progressively over the 3-wk diabetic period in conjunction with a progressive rise in blood pressure (Fig. 4). This response in the D+L rats confirmed our previous find- ings (15), and the results in the D+LB rats in this study suggest that the progressive renin stimulation depends greatly on the sympathetic nervous system.

Blood glucose values were not different between the four groups of rats during the control period and averaged 6.7 ± 0.2, 6.9 ± 0.1, 7.0 ± 0.2, and 6.6 ± 0.2 mmol/l in the D, D+B, D+L, and D+LB rats, respectively. After the induction of diabetes, blood glucose levels increased significantly in all four groups with a

![Fig. 2. Change in MAP during the 3-wk diabetic period in diabetic rats.](http://ajpregu.physiology.org/)

![Fig. 3. Glomerular filtration rate during a 3-wk diabetic period in diabetic rats with vehicle and no other treatment (D, n = 9), with propranolol and terazosin (D+B, n = 6), with L-NAME (D+L, n = 8), or with L-NAME, propranolol, and terazosin (D+LB, n = 7).](http://ajpregu.physiology.org/)
trend for lower levels in the L-NAME-treated diabetic rats. By day 20 of the diabetic period, blood glucose levels averaged 25.7 ± 1.0, 24.5 ± 3.6, 18.9 ± 3.1, and 20.0 ± 2.9 mmol/l in the D, D+B, D+L, and D+LB rats, respectively. In the D and D+B rats, the insulin infusion doses used to prevent severe hyperglycemia averaged 0.6 ± 0.2 and 0.5 ± 0.2 U/day in the first week and then stabilized at an average of 0.4 ± 0.1 and 0.3 ± 0.1 U/day for the last week of diabetes, respectively. Interestingly, the insulin infusion dose was significantly higher in the D+L and D+LB rats initially, averaging 3.2 ± 0.5 and 3.7 ± 0.4 U/day for week 1 (P < 0.001), but was not different from the D rats during week 3 of diabetes, averaging 0.5 ± 0.2 and 0.8 ± 0.5 U/day, respectively. Food intake was not different between the four groups during the control period and averaged 21 ± 2, 20 ± 2, 21 ± 1, and 20 ± 2 g/day for the D, D+B, D+L, and D+LB rats, respectively. After the induction of diabetes, all four groups increased eating significantly; by day 10, food intake was 36 ± 1, 33 ± 2, 33 ± 2, and 32 ± 1 g/day, respectively (P < 0.001). During the last week of diabetes, food intake in the D+L and D+LB rats decreased toward control levels.

Plasma protein concentrations increased significantly during the diabetic period in all groups and by day 20 of diabetes had increased by 10 ± 2%, 11 ± 3%, 7 ± 5%, and 10 ± 2% in the D, D+B, D+L, and D+LB rats, respectively (P < 0.001) with no between-group differences. Hematocrit did not change significantly during the diabetic period in any group.

The blood pressure responses to bolus infusions of phenylephrine and isoproterenol were measured before and during chronic α- and β-adrenergic receptor blockade. Throughout the diabetic period, we achieved >85% inhibition of the respective pressor and depressor responses.

DISCUSSION

Endogenous NO plays an important role in the maintenance of normal blood flow and arterial pressure under normal conditions (19, 30, 49), but the role of changes in the NO system in mediating hemodynamic disturbances associated with diabetes is unclear. This study confirms our previous observation that induction of diabetes in L-NAME-treated rats causes a significant, progressive increase in MAP that does not occur in normal diabetic rats (15) and also shows that 1) the hypertensive response is significantly attenuated in rats with chronic α- and β-adrenergic receptor blockade, and 2) hypertension in L-NAME-treated diabetic rats is related to increased renin-angiotensin system activity and to an attenuation of diabetes-associated hyperfiltration.

There is evidence for both an increase (10, 16, 31, 36) and a decrease (27, 36) in the activity of the NO system in the diabetic state. Evidence for increased NO production under hyperglycemic conditions is shown, for example, by the increase in NO release in endothelial cells exposed to high glucose levels (9, 16, 18). Furthermore, indices of NO production such as nitrate and nitrite levels are elevated in experimentally induced models of diabetes (3, 31). This is in contrast to the findings of unchanged nitrate levels in diabetic patients (40), and NO production has been reported to decrease in mesangial and vascular smooth muscle cells maintained under conditions of high glucose levels (33, 45). The inconsistencies in these various findings might be explained by experimental conditions, such as the effects of differing glucose concentrations, tissue-specific responses, or differences in the duration of diabetes. Indeed, a recent study by Pieper (36) demonstrated a triphasic response of endothelium-dependent relaxation (that is, enhanced, unchanged, and impaired relaxation) that depends on the duration of diabetes. It also should be noted that NO production may not necessarily reflect NO activity during diabetes, because enhanced free radical production may lead to decreased NO activity despite increased synthesis (16). A potential confounding influence of streptozocin-induced side effects (such as endothelial toxicity) in our study also could be considered, but is not likely, because we have shown that streptozocin treatment has no hypertensive effect if normoglycemia is maintained with intravenous insulin replacement (4, 5, 7). Unpublished pilot studies associated with our previous report (15) also showed that there was no different response to L-NAME in streptozocin-treated rats as long as normoglycemia was maintained with insulin replacement, and we also did not measure an effect of streptozocin on endothelium-mediated vasodilation (4). Thus, although the issue of how diabetes affects the NO system is far from resolved, it is clear from the current study and our previous results (15) that the presence of a functional NO system is essential to prevent marked increases in arterial pressure after the onset of diabetes. In addition, the data from this...
study suggest that this may be due to NO counteracting the activity or influence of the sympathetic nervous system.

It is not clear how the sympathetic nervous system contributed to the severe hypertension in the L-NAME-treated (D+L) diabetic rats, but many reports suggest that NO suppresses activity of the sympathetic nervous system and that L-NAME hypertension in general has a significant sympathetic component (11, 17, 23, 29, 47, 48). Thus the effectiveness of adrenergic blockade in this study could have been due to these direct relationships with the NO system and unrelated to the diabetic treatment. The inability of L-NAME to increase MAP in the α- and β-blocked rats during the prediabetic control period (see Fig. 1, D+LB rats) in fact is consistent with the important role of the sympathetic nervous system in L-NAME hypertension. Moreover, arterial pressure remained relatively normal in those rats and tracked right along with the normal diabetic rats (see Fig. 1) for the first 1.5 wk of diabetes. However, MAP in the D+LB rats then began to increase significantly, and it is very interesting that the pattern of the MAP change in those rats was similar to that in the D+L rats (see Fig. 1). The explanation for this pattern is not clear, but because it happened in the D+L and D+LB rats, it may be due to a relationship between diabetes and NO synthesis inhibition that is independent of the sympathetic nervous system. However, the amplitude of that relationship was cut approximately in half by adrenergic receptor blockade as is shown by the changes in MAP from the prediabetic baseline (the average of the last 3 days before the onset of diabetes) in D+LB versus D+L rats, which implicates the sympathetic system in at least an important modulatory role.

Thus our data are consistent with reports that the sympathetic nervous system contributes to L-NAME hypertension in general; however, that relationship alone does not appear to explain the effect of α- and β-blockade to attenuate hypertension in L-NAME-treated diabetic rats. One mechanism through which adrenergic receptor blockade could have exerted its effect on blood pressure is through decreasing sympathetic stimulation of renal tubular sodium reabsorption and renal vasoconstriction (14); however, there were no significant differences in sodium excretory patterns or GFR in this study that could be attributed to the adrenergic receptor blockade. On the other hand, the sympathetic nervous system also is a powerful controller of renin secretion (26), and the significant effect of adrenergic blockade on both PRA and blood pressure in this study points to the renin-angiotensin system as the main effector of the sympathetic system in controlling blood pressure at the onset of diabetes.

In this study and in two previous studies (5, 15), we measured significant increases in PRA during the first week of diabetes. This study also confirms our previous observation (15) and a report by Kikkawa and colleagues (21) that the response is biphasic. However, when diabetes is induced in L-NAME-treated rats, PRA continues to increase during weeks 2 and 3 of diabetes rather than return to control levels (see Fig. 4 and Ref. 15). This implicates ANG II in the hypertensive response, and because sympathetic blockade restored the biphasic renin response in the L-NAME-treated diabetic rats, this indicates that the sympathetic nervous system is required for the progressive stimulation of the renin-angiotensin system in the diabetic rats with NO synthesis inhibition. We cannot tell from this study, however, whether sympathetic activity increased or whether there was increased dependence on the sympathetic nervous system at the onset of diabetes.

Degeneration of the autonomic nervous system is a component of diabetic neuropathy, but although there is evidence for early effects on neurons (8, 42), it appears that such actions may not translate directly to significant changes in nerve structure or function during the early time period on which this study focused (32, 41, 42). Thus, with numerous reports during the last 20 years that implicate glucose as a stimulator of the sympathetic nervous system (12, 24, 28, 38, 39), it remains possible that sympathetic activity increased. On the other hand, it also is known that sympathetic blockade has a powerful ability to attenuate the effectiveness of other stimuli for renin secretion (13, 22, 26). The increase in renin-angiotensin system activity that we measured, therefore, may be due to nonsympathetic mechanisms, but the response may have been blunted in the absence of basal sympathetic input. In fact, it is highly likely that other mechanisms are operative to at least some extent. For example, acute intrarenal hyperglycemia has been shown to increase renin secretion due to stimulation of proximal tubular sodium reabsorption (46). Thus the sympathetic nervous system was required for the progressive increase in PRA, and that action seemed to account for most of the blood pressure effect of adrenergic blockade. However, additional experiments are needed to determine whether its effect on renin secretion was permissive or stimulatory.

The interrelationship between NO, the sympathetic nervous system, and the renin-angiotensin system in this experimental setting, therefore, might be that the onset of diabetes stimulates renin secretion and NO synthesis, and that an action of NO to suppress sympathetic nervous system activity (11) is important to prevent runaway increases in renin-angiotensin system activity. If the sympathetic nervous system actually is stimulated by onset of diabetes, that also would provide a driving force for renin secretion and still remain a target for a suppressive action of NO. Regardless of the degree of sympathetic stimulation, these data suggest that with the ability to synthesize NO blocked, diabetes causes an exaggerated increase in blood pressure that is strongly associated with a sympathetic-dependent increase in renin secretion. This hypothesis, however, does not explain why blood pressure still increased significantly in the D+LB rats.

One possible explanation is that adrenergic receptor blockade was not 100%; however, it also is interesting to consider the changes in GFR among the different groups. Figure 3 shows the expected hyperfiltration in
the normal diabetic rats (open bars); the diabetic rats with adrenergic blockade, except for an unexplained dip during week 2, had very similar GFRs. Both L-NNAME-treated groups, however, showed significant impairment in the hyperfiltration response, and there were no differences between the two groups. We reported a similar response for the D+L treatment in our previous study (15), which is consistent with reports that diabetic hyperfiltration depends significantly on NO (10, 25, 31). Thus the inability to increase GFR at the onset of diabetes is associated with increased blood pressure. Although increased GFR is a primary determinant of diabetic renal injury, it also increases renal excretory capability and thereby (in the short term) would tend to exert a blood-pressure-lowering influence. We can speculate, therefore, that a component of the hypertension in the D+L and D+LB groups is due to attenuated hyperfiltration, but it is clear that additional experiments are needed to explore this and other possibilities.

Another relationship that should be considered further is the potential link between NO and insulin resistance. The changes in insulin dose, blood glucose, and food intake suggest that the L-NNAME-treated rats were more insulin resistant than the other diabetic rats at the onset of diabetes, and that insulin sensitivity improved during the 3-wk diabetic period. These changes are consistent with our previous findings (15) and with the observation that NO can affect glucose uptake (1); however, they are opposite to the hypothesized link between insulin resistance and blood pressure, because the blood pressure increase was greatest later in the diabetic period. Thus, later in the study, when the MAP and GFR differences associated with L-NNAME treatment were most apparent, the differences in apparent insulin sensitivity were minimized. Although this suggests a dissociation between insulin sensitivity and hemodynamic variables, cause-and-effect conclusions cannot be drawn from this study.

These results suggest, therefore, that the sympathetic nervous system may play an active role at the onset of diabetes in promoting hypertension directly or via stimulation of renin secretion, or it simply may be required for other stimuli of renin secretion to be effective. There also may be a role for NO to directly control renin secretion in addition to its potential action to suppress the sympathetic nervous system. Despite these uncertainties, the results of this study confirm our previous report that NO synthesis is required at the onset of diabetes to prevent significant increases in MAP, and also confirm the link between a runaway increase in PRA and the hypertension in those rats. In addition, these results also suggest that the sympathetic nervous system is necessary for diabetes and L-NNAME to exert a potentiated hypertensive effect. Understanding the mechanisms that link NO, the sympathetic nervous system, and the renin-angiotensin system in the control of blood pressure in diabetes, however, will require considerable further study.

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REFERENCES