Nitric oxide may be required to prevent hypertension at the onset of diabetes

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Fitzgerald, Sharyn M., and Michael W. Brands. Nitric oxide may be required to prevent hypertension at the onset of diabetes. Am J Physiol Endocrinol Metab 279: E762–E768, 2000.—Nitric oxide (NO) plays an important role in the regulation of vascular tone, and evidence suggests that endothelial-dependent relaxation, possibly mediated via NO, is impaired in diabetes. However, the role of the endothelium in arterial pressure control early in diabetes, before dysfunction develops, is not known. This was evaluated in the present study by comparing the responses to induction of diabetes in vehicle-treated rats (D, n = 7) vs. rats chronically treated with N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME; D+L, n = 8). A nondiabetic group also was treated with L-NAME (L, n = 7) to control for L-NAME effects over time, independent of diabetes. After baseline measurements, rats were given either vehicle or L-NAME (10 \mu g \cdot kg\(^{-1}\) \cdot min\(^{-1}\) iv) infusion throughout the experiment. Six days later, streptozotocin (60 mg/kg iv) was administered, followed by a 3-wk diabetic study period. Induction of diabetes in the D+L rats caused a marked and progressive increase in mean arterial pressure throughout the diabetic period, averaging ~70 mmHg greater than in the D rats and ~20 mmHg greater than in the L rats. Glomerular filtration rate and renal plasma flow tended to increase during diabetes, but this trend was reversed in the D+L rats. In addition, plasma renin activity increased in the D and D+L rats during week 1 of diabetes but then returned to control in the D rats, while continuing to increase in the D+L rats. These results suggest that, in the early stages of diabetes, NO synthesis is important to prevent hypertension from developing, possibly through actions to maintain glomerular filtration and suppress renin secretion.

N\textsuperscript{G}-nitro-L-arginine methyl ester; arterial pressure; glucose; angiotensin II; vasodilation

THERE IS CONSIDERABLE EVIDENCE suggesting that endothelial function is impaired in diabetes (13, 27–29, 33). The impairment has been linked to numerous circulatory derangements in diabetes, including increased capillary permeability (37), enhanced platelet aggregation (10), and accelerated progression of diabetic nephropathy (13). In addition, loss of endothelial-dependent vasodilator capacity, mediated to a great extent by nitric oxide (NO), may cause excessive vasoconstriction in some vascular beds (7, 18, 27, 34). However, emphasis on the role of an impaired endothelium in mediating cardiovascular dysfunction during the established stages of diabetes has diverted attention, somewhat, away from the importance of a normal endothelium at the onset of diabetes.

Even under baseline conditions in normal animals and humans, NO production by the endothelium is important in maintaining normal blood flow and arterial pressure, as evidenced by the significant vasoconstrictor and hypertensive responses to NO synthesis inhibition (44). Although some data suggest that glucose may impair NO production (20), there is also evidence that NO production actually is increased in the early stages of diabetes and also is important in maintaining the increased renal blood flow (13, 22). Blockade of NO synthesis under those conditions, therefore, might be expected to cause an even greater hypertensive response, especially when the tendency for poor glycemic control to increase arterial pressure is considered (9, 23, 24). Therefore, we tested the hypothesis that, at the onset of diabetes, before there has been time for endothelial dysfunction to develop, there is increased dependence of arterial pressure and renal vascular resistance on NO synthesis.

METHODS

The experiments were conducted in 22 male Sprague-Dawley rats (345 ± 3 g, Harlan Sprague Dawley, Madison, WI). Surgery and care of the rats were conducted in accordance with National Institutes of Health guidelines with protocols that had previous approval from the Animal Care and Use Committee of the University of Mississippi Medical Center. Anesthesia was induced with pentobarbital sodium (50 mg/kg ip), and atropine (40 \mu g/rat ip) was administered to ensure an unobstructed airway. Body temperature was maintained at ~37°C by use of a servo-controlled heating pad. Under aseptic conditions, a laparotomy was performed, and a nonocclusive, polyvinyl catheter was implanted in the abdominal aorta through a puncture wound in the aortic wall made with the tip of an L-shaped 18-gauge needle. The insertion point was sealed with cyanoacrylate adhesive, and the catheter was exteriorized through the lateral abdominal wall. A femoral vein catheter was implanted through a sep-

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NITRIC OXIDE AND DIABETES

arate incision, and the tip was maneuvered into the inferior vena cava caudal to the kidneys. All incisions were infiltrated with penicillin G procaine (300,000 U/ml) and Sensordane at closure, and the catheters were routed subcutaneously to the scapular region and exteriorized through a Dacron-covered stainless steel button sutured subcutaneously over the scapula.

The rats were allowed to recover from surgery in a warmed cage. Thereafter, rats were placed in individual metabolic cages in a quiet, air-conditioned room with a 12:12-h light-dark cycle. Throughout the study, the rats received food and water ad libitum. The catheters were connected to a dual-channel infusion swivel (Instech, Plymouth Meeting, PA) mounted above the cage and were protected by a stainless steel spring that also served as a tethering device.

The arterial catheter was filled with heparin solution (1,000 USP U/ml) and connected, via the hydraulic swivel, to a pressure transducer (Cobe, Lakewood, CO) mounted on the cage exterior at the level of the rat. 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 for 4 s each minute, 24 h/day. The venous catheter was connected, also via the hydraulic swivel, to a syringe pump (Harvard Apparatus, Millis, MA) that ran continuously throughout the study. Total sodium intake throughout the experiment was maintained constant at ~3.1 mmol/day by continuous intravenous infusion of 25 ml of sterile 0.9% saline per day, combined with sodium-deficient rat chow (0.006 mmol sodium/g; Teklad, Madison, WI). A sodium-deficient diet ensured that the daily sodium intake could be controlled precisely at normal levels by the infusion, independent of food intake. The infusion was started immediately after placement of rats in their cages, and ~1 wk was allowed for recovery and acclimation before baseline measurements were made.

Experimental protocol. To test the role of NO in controlling arterial pressure at the onset of diabetes, two groups of rats were made diabetic, with one group subjected to chronic baseline measurements were made. To test the role of NO in controlling arterial pressure at the onset of diabetes, two groups of rats were made diabetic, with one group subjected to chronic baseline measurements were made. To test the role of NO in controlling arterial pressure at the onset of diabetes, two groups of rats were made diabetic, with one group subjected to chronic baseline measurements were made.

The rats were divided randomly into three groups: diabetic (D; n = 7), diabetic pretreated with L-NAME (D+L; n = 8), and normal rats pretreated with L-NAME (L; n = 7). After 5 days of baseline measurements, the control period (groupwise P < 0.001). After the induction of diabetes, there was a significant effect of treatment on the arterial pressure responses among the three groups of rats (groupwise P < 0.001, Fig. 1). Induction of diabetes in the D+L rats caused a marked and progressive increase in MAP throughout the diabetic period. In the last week of diabetes, MAP in the D+L rats averaged ~70 mmHg greater than in the D rats and ~20 mmHg greater than in the L rats. The effects may be seen more clearly as the change in pressure from control (Fig. 2). Control was an average of the last 3 days before the day on which diabetes was induced in the D and D+L rats. Also included for reference are data from a previous study (8) (NORMAL) showing the stability of MAP measured over the same time course in similarly instrumented and maintained normal rats, but with only saline vehicle infused throughout. These data show, therefore, that chronic inhibition of NO synthesis caused diabetes to induce a significant increase in MAP that was greater than the effect of L-NAME alone.

Heart rates were not different between groups during the precontrol period, and L-NAME treatment in the D+L and L rats decreased heart rate by ~25 beats/min (Fig. 1). Heart rate in the L rats remained relatively constant at that lower rate for the remainder of the experiment. There was a significant effect of treatment on the heart rate responses to the induction of diabetes in the three groups of rats (groupwise P < 0.001). The induction of diabetes caused pronounced bradycardia in the D rats, to a rate that averaged 101 ± 6 beats/min below control levels by day 20 of the diabetic period. Heart rate in the D+L rats showed a similar response initially, but after decreasing by 43 ±
3 beats/min by day 5 of diabetes, heart rate began to increase and reached levels not different from those of L rats by day 20. Thus L-NAME treatment in diabetic rats attenuated the decline in, and eventually eliminated, the bradycardic effect of diabetes.

Sodium intake was fixed at 3.1 mmol/day throughout the course of the study. During the control period, urinary sodium excretion was not significantly different among the three groups, averaging 2.4 ± 0.2, 3.1 ± 0.1, and 3.1 ± 0.3 mmol/day for the D, D+L, and L rats, respectively. However, the induction of diabetes in the D and D+L rats produced a marked increase in sodium excretion, resulting in a progressively greater sodium loss during the 3-wk diabetic period (groupwise \( P < 0.001 \)). In the D+L rats, however, there was a notable tendency for blunting of the sodium loss, although this did not reach statistical significance. At the end of the diabetic period, the cumulative sodium loss was 22.7 ± 1.5 mmol in the D rats compared with a negative sodium balance of 16.6 ± 2.2 mmol in the D+L rats (Fig. 1).

During the control period, GFR averaged 3.6 ± 0.1 ml/min in the D rats and was significantly lower in the L-NAME-treated groups, averaging 3.2 ± 0.2 and 2.9 ± 0.1 ml/min in the D+L and L rats, respectively (Fig. 3, \( P < 0.05 \)). Similarly, RPF tended to decrease after L-NAME treatment, averaging 7.2 ± 0.4 and 6.7 ± 0.1 ml/min in the D+L and L rats, respectively, compared with 8.2 ± 0.5 ml/min in the D rats during the control period. With induction of diabetes, GFR and RPF
tended to increase in the D rats, although the increase was not statistically significant. L-NAME treatment, however, not only prevented those increases during diabetes in the D+L rats but actually resulted in significant decreases in GFR and RPF during week 3 of diabetes (groupwise P < 0.05). In the L rats, these variables remained stable for the 3-wk experimental period. Thus the decrease in GFR and RPF in the D+L rats during week 3, which did not occur in the L rats, suggests there was increasing dependence on NO for maintenance of GFR during diabetes. It also is noteworthy in that regard that the decline in GFR in the D+L rats during week 3 of diabetes coincided with a significant difference in the rate of increase in MAP in the D+L vs. the L rats (Fig. 1).

L-NAME treatment tended to decrease PRA, averaging 2.49 ± 0.18 and 3.46 ± 0.32 ng angiotensin I (ANG I)·ml⁻¹·h⁻¹ in the D+L and L rats, respectively, compared with 4.13 ± 0.53 ng ANG I·ml⁻¹·h⁻¹ in the D rats, but the changes were not significant. After the induction of diabetes, PRA increased significantly in both diabetic groups by day 5, increasing by 3.00 ± 0.78 and 5.44 ± 0.88 ng ANG I·ml⁻¹·h⁻¹ in the D and D+L rats, respectively (Fig. 4, groupwise P = 0.05). In addition, PRA continued to rise in the L-NAME-treated rats, with the level in the D+L rats significantly greater than that in the L rats by day 20 (P < 0.05). In the D rats, on the other hand, PRA returned to control levels during the remainder of the diabetic period.

Blood glucose was not different among the three groups of rats during the control period, averaging 7.1 ± 0.2, 7.1 ± 0.2, and 6.9 ± 0.1 mmol/l in the D, D+L, and L rats, respectively, and it did not change in the L rats over the next 3 wk. After the induction of diabetes, blood glucose increased markedly and remained at hyperglycemic levels throughout the study, averaging 23.7 ± 0.7 and 21.3 ± 1.9 mmol/l in the D and D+L rats, respectively, for the 3-wk period. Interestingly, glucose in the D+L rats began to decrease toward control during the last week of diabetes, averaging 17.8 ± 2.4 mmol/l for the last 7 days. This was significantly lower than in the D rats (groupwise P < 0.05), and the insulin dose was decreased progressively to correct the fall. Beginning on day 3 after induction of diabetes, insulin was administered to both diabetic groups, through continuous intravenous infusion, to try and maintain fasting glucose in the range of 20–25 mmol/l. In the D rats, the insulin infusion dose averaged 0.6 ± 0.3 U/day for the first 7 days of treatment and then stabilized at an average of 0.3 ± 0.2 U/day for the remainder of the diabetic period. In the D+L rats, the insulin infusion dose was significantly higher initially, averaging 1.2 ± 0.2 U/day for the first week (groupwise P = 0.05), but was not different from the D rats during week 3, averaging 0.6 ± 0.2 U/day. Food intake was not different among the three groups during the control period, averaging 22 ± 1, 19 ± 1, and 20 ± 1 g/day for the D, D+L, and L rats, respectively, and did not change in the L rats over the next 3 wk. After the induction of diabetes, both the D and D+L rats increased their eating, and by day 14, food intake was 35 ± 3 and 27 ± 2 g/day in the D and D+L rats, respectively (groupwise P < 0.001). During the last week of diabetes, however, food intake in the D+L rats decreased toward control levels.

Plasma protein concentration tended to increase during the experimental period in all groups, but the increase (11%) was significant only in the D rats (P < 0.001). During the diabetic period, hematocrit increased in the D rats (increasing from 0.41 ± 0.00 to 0.43 ± 0.01) while decreasing in the D+L rats (decreasing from 0.43 ± 0.00 to 0.40 ± 0.01).

**DISCUSSION**

These results show that blockade of NO synthesis causes a significant increase in MAP at the onset of diabetes. Although we have shown previously that there is a consistent trend for MAP to increase with poor glycemic control in diabetic rats (7, 9), the increase in arterial pressure was three- to fourfold greater with NO synthesis inhibition. Associated closely with the increase in MAP, the L-NAME-treated diabetic rats also showed a significant impairment in their ability to maintain GFR, and a much greater increase in PRA. Thus at the onset of diabetes, NO synthesis is essential to prevent hypertension from developing, perhaps in part because of actions that maintain glomerular filtration and suppress renin secretion.

Activity of the NO system in diabetes is somewhat controversial, with studies providing evidence for either an increase (12, 14, 22, 28) or a decrease (3, 28, 35) in the synthesis of NO in the diabetic state. Likewise, endothelial-mediated vasodilation has been reported to be impaired in diabetic humans (17, 21) and animals (1, 27), but not all studies report impairment (7, 15, 31). In addition, NO synthesis may not accurately reflect NO activity in various stages of diabetes, because the level of free radical production can greatly affect activity, even causing a decrease in NO activity.

**Fig. 4.** Plasma renin activity during a 3-wk experimental period in the D, D+L, and L rats. Values are means ± SE; n = 7. *P < 0.05 vs. control period.
despite increased synthesis (12, 14). Thus, although the endothelium may function normally, particularly at the early stages of diabetes, a dysfunctional response to endothelium-dependent vasodilatory stimuli may occur. The reason for the discrepancies between observations in different studies is not known but could be the influence of ambient glucose concentrations at the time of the study, tissue specific responses, or differences in the duration of diabetes at the time of testing. This study was not designed to address this complex issue, but we have shown that the vasodilatory response to acute acetylcholine infusion is not impaired during the 1st wk of diabetes in this model (7), and the present results suggest, furthermore, that the ability to synthesize NO is important to prevent significant increases in arterial pressure during the early stages of diabetes.

The observation that blockade of NO synthesis caused MAP to increase significantly at the onset of diabetes does not exclude the possibility that mechanisms other than removal of the vasodilator influence of NO contributed to the hypertension. A role of the sympathetic nervous system, for example, is suggested by the heart rate response to NO synthesis inhibition in the diabetic state. The bradycardia in the diabetic rats (D) is consistent with suppression of the sympathetic nervous system, and the increase in heart rate in the diabetic rats treated with L-NAME (D+L) suggests that activation of the sympathetic nervous system occurred in the absence of NO. Indeed, it has been demonstrated that NO can suppress sympathetic nervous system activity (43). Thus inhibition of sympathetic nervous system activity may have contributed to NO's action to prevent increases in arterial pressure at the onset of diabetes, and likewise, increased sympathetic activity may have mediated a significant component of the marked hypertension in the L-NAME-treated diabetic rats (D+L).

Another, although not necessarily unrelated, mechanism whereby NO could affect arterial pressure at the onset of diabetes is through the control of renal vascular resistance. Our previous studies have shown that the onset of diabetes in rats is associated with a modest increase in arterial pressure (9) and a marked decrease in 24 h/day-measured hindquarter blood flow (7), consistent with peripheral vasoconstriction. One possibility, therefore, is that renal hyperfiltration and natriuresis serve as mechanisms to counteract an increase in arterial pressure. These renal responses could also be acting to balance a shift in the pressure-natriuresis relationship, but because glucose-mediated osmotic diuresis contributes to the renal sodium loss in diabetes, it is not clear how the induction of diabetes actually shifts pressure natriuresis. Regardless, however, it is tempting to speculate that NO plays at least a permissive role in the development and persistence of the hyperfiltration and natriuresis, and that these actions help counteract a pressor stimulus associated with diabetes in rats (9). If so, then blockade of NO synthesis during diabetes should attenuate the renal responses and increase arterial pressure. The natriuresis showed signs of being attenuated in the D+L rats compared with the D rats, but the differences were not statistically significant. GFR, on the other hand, tended to increase in the diabetic rats but did not increase with induction of diabetes in the L-NAME-treated rats, and arterial pressure increased more in the latter group. Moreover, GFR actually decreased significantly during the latter stages of this study in the D+L rats, and this was associated with a further increase in the rate of the arterial pressure rise. Thus, although other factors likely are important in determining the renal responses, sodium excretion in particular, the ability to increase or maintain GFR at the onset of diabetes appears to be dependent on NO synthesis and may be a mechanism through which NO prevents arterial pressure from increasing.

The control of GFR in diabetes is not well understood, even though it is recognized that at the early stages of both clinical and experimentally induced diabetes there is a significant increase in GFR (25, 41). The maintenance of increased GFR in the face of natriuresis suggests that there has been a resetting or impairment in the feedback control of GFR via the macula densa mechanism (6), and NO could affect these relationships through several mechanisms. There is good evidence that NO blunts tubuloglomerular feedback (TGF) (5, 40), and an effect of diabetes to increase NO production is consistent with the blunting of TGF associated with diabetes (6, 38). Because this change in TGF has been proposed to explain, at least in part, the hyperfiltration in diabetes (6, 25, 41), the failure of GFR to increase in the diabetic rats with NO synthesis blocked is consistent with this mechanism. However, increased NO production at the onset of diabetes also could raise GFR through direct vascular actions (22). Likewise, other factors could mediate the increase in GFR, and basal NO production may be required only to offset the influence of vasoconstrictor stimuli; however, this study cannot differentiate between these possible mechanisms.

The actions of NO on the renal vasculature and macula densa also mediate much of its effect on renin secretion (19), and our results suggest that changes in renin secretion may have affected the arterial pressure responses to the onset of diabetes as well. This is consistent with the findings of Miller et al. (24), who demonstrated that short periods of hyperglycemia in early type 1 diabetic patients increased renin secretion and also increased the dependence of arterial pressure on angiotensin II (23). Similarly, in our study, PRA increased in both diabetic groups during the 1st wk of diabetes. As diabetes progressed, however, PRA returned to control in the D rats but continued to increase in the D+L rats. This suggests that increased NO production was responsible for the decrease in renin secretion back to control levels during the last 2 wk of diabetes in the D rats. The mechanism for the initial increase in PRA is not clear but appears to be independent of NO, because it increased in both the D and D+L rats. In addition, that stimulus seemed to be maintained, because PRA in the D+L rats was signif-
icantly greater than in the L rats on day 20 of the experimental period. Although the mechanism for the effect of NO on renin secretion is not known, the renin-suppressing effect of NO during the last 2 wk of this study was associated closely with the ability to prevent arterial pressure from increasing during dia-

Other important variables that must be considered in mediating the effects of NO synthesis inhibition in this study relate to food intake and glucose utilization. All diabetic rats received the same dose of STZ, and porcine insulin was added to the infusate as needed to keep all diabetic rats between 20 and 25 mmol/l. By about the 5th day of diabetes, a stable insulin dose per rat was achieved, and blood glucose in both diabetic groups was very similar. However, during the 3rd wk of diabetes, blood glucose began to decrease toward 17 mmol/l in the D+L diabetic rats, and they required a decrease in the insulin dose to try and keep glucose in the desired range. This suggests that insulin sensitiv-

pressure. That effect, however, although beneficial for short-term arterial pressure control, could contribute to accelerated progression of renal injury in diabetes. Further study will be needed to test these hypotheses and to determine the role of the specific NOS isoforms in mediating these effects.

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