Plasma and Vascular Tissue Arginine Are Decreased in Diabetes: Acute Arginine Supplementation Restores Endothelium-Dependent Relaxation by Augmenting cGMP Production

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ABSTRACT
Arginine is a precursor amino acid for the synthesis of nitric oxide by nitric oxide synthase. A defect in arginine supply could regulate nitric oxide-mediated, endothelium-dependent relaxation. In this study, we evaluated the effect of supplementation with L-arginine given in vitro on both functional relaxation and cGMP generation in response to acetylcholine in the streptozotocin-induced diabetic rat aorta. The concentration of arginine in plasma and aortic tissue were both decreased by diabetes. Acute incubation in vitro with L-arginine augmented the impaired relaxation to acetylcholine in diabetic rings although not altering relaxation in control rings. L-Arginine also enhanced relaxation to acetylcholine in diabetic rings incubated in the presence of either indomethacin or tetraethylammonium to inhibit cyclooxygenase activity and potassium channel activity, respectively. Acetylcholine-stimulated cGMP generation (which was blocked by L-nitroarginine) was diminished in diabetic rings compared with control rings. L-Arginine restored cGMP in diabetic rings (with but not without endothelium) to levels similar to control rings. L-Arginine did not alter cGMP generated by nitroglycerin. Incubation with L-arginine had no effect on acetylcholine-stimulated cGMP generation in control rings (with and without endothelium). These data suggest a potential intracellular substrate deficiency in nitric oxide production by diabetic endothelium which can be overcome acutely in vitro by provision of substrate for nitric oxide synthase.

Chronic experimental diabetes mellitus produces impairment of endothelium-dependent relaxation (see reviews: Pieper and Gross, 1991; Cohen 1993; Kamata et al., 1992). This defect has been confirmed in type I (Johnstone et al., 1993; McNally et al., 1994) and type II (McVeigh et al., 1992) diabetic patients. There is growing evidence from experimental models that alterations in several independent pathways may contribute to impaired endothelium-dependent relaxation of diabetic blood vessels and that a single factor may be inadequate to uniformly explain dysfunctional relaxation in all instances and at various stages of the disease. These factors include: 1) co-release of an endothelium-derived constricting factor derived from the cyclooxygenase pathway (Tesfamariam et al., 1989; Mayhan, 1989); 2) increase in protein kinase C (Pelligrino et al., 1994); 3) increased quenching of NO by advanced glycosylation end-products (Bucala et al., 1991); 4) decreased NO activity because of interaction with increased production of oxygen radicals (Pieper et al., 1992, 1996a; Diederich et al., 1994; Tesfamariam and Cohen, 1992); and 5) abnormal NO synthase activity caused by inadequate co-factor (Pieper, 1997). One factor that has received scant attention is the possibility that substrate supply for efficient NO production by NO synthase in the diabetic endothelium may be compromised.

NO or a closely related molecule accounts for a major portion of the endothelium-dependent relaxation produced in a variety of blood vessels. NO is synthesized in endothelial cells by enzymatic conversion by NO synthase of the precursor amino acid, ARG, to form NO plus citrulline. Thus, disease states in which ARG levels are reduced may produce inadequate levels of NO and impaired endothelium-dependent relaxation. In this regard, the plasma concentration of ARG has decreased in experimental diabetic animals (Mans et al., 1987) as well as in diabetic patients (Grill et al., 1992; Hagenfeldt et al., 1989).

In previous studies from our laboratory, we observed that L-ARG given acutely in vitro (Pieper and Peltier, 1995; Pieper et al., 1995) or in vivo (Pieper et al., 1996b) restored endothelium-dependent relaxation to ACH in diabetic rat aorta
without altering responses to nitroglycerin. These results are similar to the improved endothelium-dependent relaxation elicited by L-ARG in experimental models of cardiomyopathy (Mayhan and Rubinstein, 1992), chronic hypoxia (Eddahibi et al., 1992; Carville et al., 1993), balloon-induced endothelial injury (Hamon et al., 1994; Tarry and Makhoul, 1994), hypercholesterolemia/atherosclerosis (Cooke et al., 1991; Kuo et al., 1992; Boger et al., 1995) and hypertension (Kitazono et al., 1996) and in hypercholesterolemia in human patients (Creager et al., 1992; Clarkson et al., 1996).

In all of these disease models, the mechanism for the improved relaxation induced by ARG treatment was assumed to be caused specifically by enhanced NO-mediated and enhanced cGMP-dependent production. Despite these assumptions, no one has specifically addressed this assumption in any of the models listed above, including diabetes. We believe these assumptions require further examination for several reasons. First, NO-mediated relaxation has been shown to act via cGMP-independent pathways and to elicit relaxation via K$^+$-sensitive channel activation (Cohen and Vanhoutte, 1995). In some instances ARG analogs inhibit dilation mediated by certain K$^+$-sensitive vasodilators (Kontos and Wei, 1996). Thus, it is possible that ARG may enhance relaxation via a K$^+$-sensitive mechanism. Furthermore, there is even some question whether ARG exclusively enhances relaxation via NO because ARG analogs that inhibit NO synthase also blunt prostaglandin-mediated relaxation (Koller et al., 1993) and indomethacin blocks L-ARG-enhanced dilation in hypertensive rats (Riedel et al., 1995).

Because of these new uncertainties, we reexamined the hypothesis that l-ARG treatment of diabetic blood vessels increases endothelium-dependent relaxation via a NO/cGMP-dependent pathway. First, we sought to determine whether reduced plasma ARG levels in diabetes lead to frank reductions in vascular tissue stores of ARG because this had not been evaluated previously. Second, we determined whether this was associated with decreased NO production as assessed by both functional relaxation and cGMP generation. Third, we performed additional experiments in the presence of indomethacin or TEA to circumvent potential pathways of enhanced L-ARG-induced relaxation via prostanoid or K$^+$-sensitive pathways. Finally, we sought to determine whether the improvement by L-ARG supplementation in endothelium-dependent relaxation of diabetic blood vessels could be explained by an actual improvement in NO/cGMP production.

Materials and Methods

Adult male Sprague-Dawley (Sasco, Inc., Madison, WI) rats (approximately 90 days of age) were anesthetized with an intraperitoneal injection of 100 mg/kg Ketaset. Diabetes was induced in anesthetized animals by an intravenous tail-vein injection of streptozotocin (55 mg/kg in 0.1 M citrate buffer, pH 4.5). A drop of tail blood was obtained at 1 week after administration of streptozotocin to verify hyperglycemia by use of a glucometer. Diabetic and age-matched control rats were housed for 2 months before experiments were performed.

On the day of experimentation, rats were anesthetized with 65 mg/kg sodium pentobarbital. Descending thoracic and abdominal aortae were carefully isolated, removed and placed in 4°C Krebs' bicarbonate buffer. The aortic segments were carefully cleaned of fat and loose connective tissue and sectioned into 3-mm-long rings. In all instances, care was taken to avoid stretching and contact with the luminal surface to avoid damage to the endothelium during isolation.

Isolated vascular ring experiments. Thoracic aortic rings were suspended between parallel hooks in 10-ml tissue baths which were thermoregulated at 37°C in a Krebs-Henseleit medium as described previously (Piper and Peltier, 1995; Piper et al., 1995). The medium was gassed with 95% O$_2$-5% CO$_2$ to maintain pH at 7.4. Aortic rings were equilibrated and resting tension was set at an optimal level of 2.0 g for both control and diabetic rings based on length-tension studies. Changes in isometric tension were recorded on a Gould TA6000 (Gould Instruments, Oxnard, CA) recorder via Radnoti (Radnoti Instruments, Monrovia, CA) force-displacement transducers. At the completion of each experiment, the rings were blotted dry and weighed, and the lengths were measured to calculate tension as normalized for cross-sectional areas based on the formula: cross-sectional area (mm$^2$) = weight (mg) × length (mm) × density with the density of vascular tissue being 1.05 mg/mm$^3$ (Abbe et al., 1990).

Individual vascular function protocols. Each ring was exposed to increasing concentrations of NE to generate concentration-response curves. Stock solutions of NE contained 20 nM ascorbate to prevent autooxidation. After generating NE contraction-response curves, each ring was serially washed to base-line tension and equilibrated. Rings were then contracted with a submaximal NE concentration which elicited approximately 70% of the maximum response. The NE concentration was usually 1 μM but was varied, if necessary, to achieve equipotent agonist activity.

At the plateau of contraction, relaxation responses to cumulative concentrations of the endothelium-dependent vasodilator, ACH. We have previously shown that relaxation to ACH is virtually blocked in both control and diabetic rat aorta by NO synthase inhibitors (Piper and Peltier, 1995; Piper et al., 1995). For these studies, the rings were challenged once with ACH to verify that responses between individual rings from the same animal were similar. Rings were then washed serially, reequilibrated and incubated with 3 mM L-ARG for 45 min. Rings were then contracted with NE followed by a second ACH challenge. With this technique, we have previously shown similar responses between first and second challenges in both control and diabetic aortic rings (Piper and Peltier, 1995; Piper et al., 1995). This technique allows any potential differences in intravessel reactivity to be minimized by verification before exposure to various drug interventions. The experiments with L-ARG were also conducted in the presence of 10 μM indomethacin or 1 mM TEA to eliminate the possibility that ARG produced enhanced relaxation via a cyclooxygenase-dependent or K-channel-dependent pathway, respectively.

Amino acid analysis. At the time of experimentation, plasma for arginine analysis was taken from a random subgroup of control and diabetic rats. All samples were extracted 1:1 in 35% (wt/vol) sulfosalicylic acid dihydrate. After mixing and centrifugation, the supernatant was mixed 1:1 with lithium-D buffer before analysis.

Abdominal aorta was taken from a subset of animals in which the descending thoracic aorta was used for functional analysis. The fresh vascular tissue was homogenized in ground-glass microhomogenizing tubes in 800 μl of 0.5 N perchloric acid and centrifuged. An aliquot of supernatant (700 μl) was mixed with 196 μl 2 M potassium carbonate, centrifuged and frozen at −20°C for amino acid analysis. Plasma and tissue arginine (including ARG) were examined with a Beckman 6300 amino acid analyzer (Palo Alto, CA).

cGMP studies. Thoracic aortic rings from rats not used for functional studies were equilibrated in phosphate-buffered saline for 45 min. Rings with endothelium were either exposed to the following: 100 nM NE alone for 10 min plus 10 μM ACH (in the absence or presence of 100 μM L-NA and in rings with or without endothelium), or NE plus ACH in rings pretreated for 45 min with 3 mM d-ARG or L-ARG (in rings with or without endothelium). The reaction was terminated 1 min after the addition of buffer control or ACH by freezing with liquid nitrogen and homogenizing in trichloroacetic acid followed by extraction in water-saturated ether. In a few se
lected experiments, 100 μM L-NA was given during the last 20 min of incubation to block NO synthase. For control purposes, the effects of L-ARG were also studied in diabetic rings that were stimulated with 30 μM nitroglycerin for 2 min before terminating the reaction. cGMP was determined by radioimmunoassay with a commercial diagnostic kit (PerSeptive Diagnostics, Cambridge, MA).

**Statistical analysis.** Data are expressed as the mean ± S.E.M. Data were analyzed by analysis of variance followed by Fisher’s projected least-squares difference test for multiple mean comparisons or unpaired t test for comparisons of two group means or paired t test for comparisons of two group means in a pretest/posttest format. A value of P < .05 was set to denote statistical significance.

**Results**

**Body weight and blood analysis.** A total of 128 rats were used for this study. Body weight of control rats (total n = 60) increased from an initial weight of 387 ± 5 g to 532 ± 8 g at the completion of the study. In contrast, diabetic rats (total n = 68) weighed 388 ± 5 g initially and 347 ± 9 g at the end of the study. Blood glucose levels of diabetic animals were significantly increased (P < .001) above those of control animals at 1 week (i.e., 370 ± 9 vs. 80 ± 4 mg/dl) and at the end of the study (i.e., 385 ± 9 vs. 83 ± 5 mg/dl).

Diabetes produced a significant decrease in the plasma concentration of ARG (fig. 1, upper panel). In addition to diminished plasma ARG concentration in diabetic animals, the ARG content of aortic tissue was diminished significantly by diabetes (fig. 1, lower panel). Incubation of diabetic aortic segments with 3 mM L-ARG increased tissue arginine content by approximately 20-fold (i.e., to 10.6 ± 0.2 nmol/mg tissue, n = 4 determinations).

**Vascular relaxation.** Initial studies were performed in which the mean relaxation response to ACH for all rings was averaged for each animal and the mean for each group calculated. ACH produced a concentration-dependent relaxation in both control and diabetic aortic rings (fig. 2). The relaxation produced in diabetic rings was impaired relative to rings from age-matched control animals. The addition of L-ARG did not alter the relaxation to ACH in control rings (fig. 2, upper panel), but significantly improved the attenuated relaxation observed in diabetic rings (fig. 2, lower panel). The relaxation to ACH in L-ARG-treated diabetic rings was not different from that observed in untreated control or L-ARG-treated control rings. For example maximal relaxation was significantly improved by L-ARG compared with untreated diabetic rings (i.e., 86 ± 3% and 67 ± 3%, respectively; P < .01) which was not significantly different from untreated and treated control rings (i.e., 97 ± 3% and 101 ± 6%, respectively). Treatment with L-ARG also did not alter pD2 for ACH in indomethacin-treated control rings (i.e., 6.86 ± 0.12 and 6.94 ± 0.06 for rings without or with L-ARG, respectively) but did significantly change (P < .01) the pD2 for ACH in indomethacin-treated diabetic rings (i.e., 5.97 ± 0.26 vs. 6.55 ± 0.14 for rings without and with L-ARG, respectively).

In the presence of indomethacin, responses to ACH were still impaired by diabetes and pair-matched rings incubated in the presence of L-ARG also augmented relaxation to ACH (fig. 3) in diabetic rings (lower panel) but not control rings (upper panel). For example maximal relaxation was significantly improved by L-ARG compared with untreated diabetic rings (i.e., 93 ± 2% and 66 ± 6%, respectively; P < .01) which was not significantly different from untreated and treated control rings. In the presence of TEA, L-ARG enhanced relaxation to ACH in diabetic rings (fig. 4, lower panel). For example, maximum relaxation to ACH was 64 ± 6% vs. 88 ± 2% (P < .01) and the pD2 for ACH was 5.46 ± 0.30 vs. 6.29 ± 0.10 (P < .01) for rings without or with L-ARG, respectively. There was no significant change in either sensitivity to ACH in control rings by L-ARG (i.e., pD2 = 6.50 ± 0.10 and 6.72 ± 0.07 for rings without or with L-ARG, respectively, P = .16) or max-

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![Graph](attachment:image.png)

**Fig. 1.** Decrease in the plasma ARG (n = 6–7) concentration and tissue ARG (n = 5 each) content in diabetic rats. *P < .01 and **P < .01 vs. control rats.
imum relaxation (98 ± 5% and 100 ± 1% for rings without or with L-ARG, respectively) (fig. 4, upper panel).

cGMP studies. cGMP was significantly increased by ACH in control rings with endothelium compared with rings without endothelium or in rings pretreated with L-NA (fig. 5). The ACH-stimulated cGMP production was significantly attenuated in diabetic compared to control rings. Incubation with L-ARG did not alter ACH-stimulated cGMP production in control rings with or without endothelium (fig. 5). In contrast, L-ARG potentiated ACH-stimulated cGMP production in diabetic rings with endothelium but not in diabetic rings without endothelium (fig. 5). D-ARG did not alter cGMP production in response to ACH in diabetic rings (not shown). Furthermore, L-ARG did not alter NTG-stimulated cGMP production in diabetic rings (i.e., 4.8 ± 0.4 and 5.0 ± 0.6 pmol/mg protein, with and without L-ARG, respectively, n = 4 pair-matched rings each).

Discussion

In the intact cell, various factors can potentially regulate efficient NO production by NO synthase. In the present study, we provide evidence that a decrease in supply of ARG might regulate the full expression of NO release by diabetic endothelium. Accordingly, we show for the first time that both plasma and vascular tissue ARG concentrations are
decreased in diabetes and that this decrease is associated with both diminished ACH-mediated relaxation and cGMP production, a measure of NO production. Acute addition of L-ARG but not D-ARG restored relaxation and cGMP production similar to that observed in untreated and ARG-treated control blood vessels.

Although decreases in plasma ARG concentrations have been reported in experimental diabetic animals (Mans et al., 1987) and human diabetic patients (Grill et al., 1992; Hagenfeldt et al., 1989), it was not known previously whether decreases in plasma ARG concentration would be reflected in actual reductions in the ARG content of vascular tissue. This was particularly uncertain because of one report that ARG transport was actually enhanced in hepatocytes of diabetic rats (Handlogten and Kilberg, 1984); however, the liver is an important organ for the utilization of various amino acids for gluconeogenesis, etc.

In the endothelial cell, L-ARG is usually transported via the system y\(^{+}\) transporter (Greene et al., 1993; Bussolati et al., 1993). Previous estimates indicate that the \(K_m\) for L-ARG uptake in cultured bovine pulmonary artery endothelial cells was 300 \(\mu M\) (Greene et al., 1993). In contrast, a more recent study with human umbilical vein endothelial cells revealed a \(K_m\) for L-ARG transport closer to 100 \(\mu M\) and that the \(K_m\) did not change in gestational diabetes (Sobrevia et al., 1995).

In the present study, we report that plasma ARG concentration in streptozotocin-induced diabetic rats decreased from 190 to 65 \(\mu M\). It is not likely that streptozotocin produces a generalized decrease in plasma amino acids because several other amino acids are either unchanged or actually increased (Pieper and Peltier, 1995; Pieper et al., 1995, 1996b) and decreases in plasma ARG but not other amino acids have been observed in diabetic patients (Grill et al., 1992). Because the plasma ARG concentration is less than the \(K_m\) for ARG in our study, it is likely that arginine transport is not saturated and that a relative deficiency in tissue ARG levels could result. Our measurement of vascular tissue ARG levels supports this hypothesis. The decrease in tissue ARG level also is not likely to produce a generalized decrease in amino acids as a consequence of streptozotocin-induced diabetes, because we have noted either no change or increases in other amino acids in vascular tissue (unpublished observations). This observation is similar to that obtained for other amino acids in the brain tissue of streptozotocin-induced diabetic rats (Mans et al., 1987).

Furthermore, the decrease in vascular tissue ARG is not likely to be caused by intrinsic defects in the cationic amino

**Fig. 4.** Effects of addition of L-ARG on relaxation to ACH in aortic rings of control (\(n = 8\) each) and diabetic (\(n = 8\) each) rats pretreated with TEA. *\(P < .05\) and **\(P < .01\) vs. untreated.
acid transporter for ARG per se because *in vitro* studies have shown that uptake is unaltered in isolated coronary endothelial cells from the diabetic BB rat (Wu and Meininger, 1995). Although these same authors reported an actual increase in ARG content of diabetic endothelial cells despite impaired NO synthesis, it is uncertain what occurs under ambient conditions because these results were obtained in passed cells with medium containing 0.4 mM ARG. In fact, a recent study has even shown increased ARG transport in gastric glands of the diabetic rabbit at 2 to 3 days after alloxan administration (Contreras et al., 1997). These discrepancies may be accounted for by variances in models of diabetes, duration of disease, species and tissue. Thus, the relevancy of these other studies to our model is not known with certainty.

The question is raised whether this reduction of tissue ARG levels is of sufficient magnitude to be of significant consequence for NO production by endothelial cell NO synthase. It is not known what the exact molar concentration of tissue ARG was in our preparation, because our tissue values are not reported in these units; however, diabetes decreased ARG content by >40%. The intracellular ARG concentration in normal bovine aortic endothelial cells has been estimated to range from 122 μM (Mitchell et al., 1990) to 250 μM (Preik-Steinhoff et al., 1995).

The K_m for ARG for purified NO synthase has been reported to be 6 μM and the activity appears to be maximized at ARG concentrations between 30 and 100 μM (Mayer et al., 1989). The appropriate intracellular K_m for ARG for NO synthase in intact endothelial cells is unclear, because all previous estimates have been performed in cell-free systems under ideal conditions. Thus, it is likely that the intracellular K_m for ARG is likely to be higher than that reported for purified enzymes.

Other factors or conditions present under intact cell conditions could also modulate the K_m for ARG. This includes such factors as the concentration of cofactor, tetrahydrobiopterin, which in suboptimal concentrations increases the K_m for ARG (Klatt et al., 1994a, b). In this regard, the concentration of tetrahydrobiopterin is known to be reduced in the brain of diabetic rats (Hamon et al., 1989), and we have found that acute incubation with a derivative of tetrahydrobiopterin restores relaxation to ACH in diabetic blood vessels (Pieper, 1997). Thus, it is theoretically possible that supplementing cofactor may improve relaxation by increasing NO despite lower K_m content in diabetic tissue even through a process of decreasing the K_m for ARG.

We assume that the concentration of tissue ARG measured in our study is uniformly distributed between all cell types and within the endothelial cell itself, but this is not known with any certainty. Nevertheless, we cannot exclude the possibility because of the decreased overall ARG content, a localized ARG deficiency in endothelial cells could place the concentration at or near the optimal intracellular K_m for ARG for the purified constitutive NO synthase.

The fact that l-ARG improved relaxation to ACH in diabetic aortic rings is consistent with previous studies in our laboratory using two different strains of rat (Pieper and Peltier, 1995; Pieper et al., 1995) and in our genetic model of the spontaneously diabetic BB rat (Pieper et al., 1997). This agrees also with others showing improved relaxation after intravenous ARG infusion in canine coronary arteries in short-term alloxan-induced diabetes (Matsunaga et al., 1996). This suggests that the beneficial effects of arginine supplementation are independent of the model of diabetes chosen for evaluation. In contrast, topical application of L-ARG in situ failed to enhance relaxation to ACH and bradykinin in diabetic rat basilar arteries (Mayhan et al., 1996). This divergent observation could be explained by the possibility that multiple factors contribute to endothelial dysfunction in diabetes, and the contribution of individual factors might vary from one vessel type to another. In this regard, because the specific contribution to defective NO-mediated relaxation in control and diabetic basilar artery is unknown, defects in NO-independent factors may contribute to defective relaxation of diabetic rat basilar artery and may not be amenable to restoration by ARG supplementation. Indeed, Kamata and Kondoh (1996) have shown that indomethacin normalizes relaxation to ACH in diabetic rat basilar arteries.

In contrast, we have observed that ACH-mediated relaxation in both control and diabetic aortic rings of two independent rat strains is completely abrogated by incubation with NO synthase inhibitors (Pieper and Peltier, 1995; Pieper et al., 1995), which indicates that relaxation is specific for NO. This makes this preparation ideal for studying diabetes-induced defects in relaxation which are specific for NO.

An alternative and equally plausible explanation for these differences is that various factors may contribute to dysfunction at different stages of the disease. The fact that topical application of L-ARG failed to augment relaxation in the study with the basilar artery of animals with diabetes of 3 months duration is consistent with our results showing that L-ARG restores relaxation to ACH in the aortas of rats with diabetes 2 months duration but not in the aortas of rats with diabetes of 3 months duration (Pieper et al., 1995). This underscores the importance in appreciating the time-dependent progression in the etiology of diabetes-induced endothelial dysfunction and that factors that contribute to dysfunction at one stage may no longer be important or surmountable at later stages of the disease.

We also showed that the improved relaxation in response to addition of l-ARG is specific for diseased blood vessels in that responses to ACH were not improved in control rings. This salient action is stereospecific in that we previously demonstrated that d-ARG failed to augment relaxation to ACH in diabetic rings (Pieper and Peltier, 1995; Pieper et al., 1995). Furthermore, we also showed that l-ARG effects are specific for endothelium-dependent processes because responses to the endothelium-independent vasodilator NTG was unaltered by l-ARG treatment of diabetic rings (Pieper and Peltier, 1995; Pieper et al., 1995). The mechanism by which l-ARG provides this effect was not provided in our previous studies. In the present study, experiments conducted in the presence of indomethacin or TEA suggest that changes in prostanoid release or K^+ activation cannot account for the improved relaxation elicited by l-ARG in diabetic rings.

Consistent with the notion that tissue availability of ARG for synthesis of NO by diabetic aortic endothelium may be compromised is our analysis of ACH-stimulated cGMP production which was observed to be decreased in untreated diabetic aortas compared with untreated control aortas. These findings are consistent with previous measurements of decreased ACH-stimulated cGMP production in streptozotocin-induced diabetic rat aortas (Kamata et al., 1989; Miller et
Our cGMP studies extend these initial observations in several important ways. First, we demonstrated that the ACH-stimulated cGMP production is specific for NO production in both control and diabetic rat aortas because they are blocked by the NO synthase inhibitor, L-NA. Second, our data showing that L-ARG augmented ACH-stimulated cGMP production in diabetic (but not control aortas) is also consistent with a deficiency in ARG supply in diabetic vascular endothelium and cannot be accounted by supply of ARG for any NO synthase in nonendothelial tissue. Third, the additional studies in which d-ARG failed to alter cGMP production in diabetic aortas indicate that this is stereospecific. Fourth, the lack of effect of L-ARG on cGMP generated by NTG suggests that this cannot be explained by an L-ARG-induced change in guanylate cyclase reactivity. To our knowledge the present study is the first report to show improved cGMP production in diabetic blood vessels by any type of pharmacological intervention and the first to show that the action of L-ARG to improve endothelium-dependent relaxation in any diseased model is specific for enhanced cGMP generation.

Finally, it has been hypothesized that abnormal endothelial function in patients with insulin-dependent diabetic mellitus may be caused by a defect in ARG-derived NO synthesis (Calver et al., 1993). Accordingly, L-ARG given to diabetic patients might increase NO production. Indeed, there is an isolated report in which L-ARG augmented plasma concentrations of nitrite in patients with non-insulin-dependent diabetes (Das et al., 1993). Unfortunately, it is unknown whether healthy individuals respond similarly to L-ARG.

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References


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