Original Article

ACE Inhibitor or Angiotensin II Receptor Antagonist Attenuates Diabetic Neuropathy in Streptozotocin-Induced Diabetic Rats

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ACE inhibition and/or blocking of the angiotensin II receptor are recognized as first-line treatment for nephropathy and cardiovascular disease in diabetic patients. However, little information is available about the potential benefits of these drugs on diabetic neuropathy. We examined vascular and neural activity in streptozotocin-induced diabetic rats that were treated for 12 weeks with enalapril, an ACE inhibitor, or L-158809, an angiotensin II receptor blocker. A prevention protocol (group 1) as well as three intervention protocols (treatment was initiated after 4, 8, or 12 weeks of diabetes [groups 2, 3, and 4, respectively]) were used. Endoneurial blood flow and motor nerve conduction velocity (MNCV) were impaired in all groups of untreated diabetic rats. In group 1, treatment of diabetic rats with enalapril or L-158809 partially prevented the diabetes-induced decrease in endoneurial blood flow and MNCV. In groups 2–4, intervention with enalapril was more effective in reversing the diabetes-induced impairment in endoneurial blood flow and MNCV than L-158809. The superoxide level in the aorta and epineurial arterioles of diabetic rats was increased. Treatment of diabetic rats with enalapril or L-158809 reduced the superoxide level in the aorta in all groups but was less effective in epineurial arterioles. Acetylcholine and calcitonin gene–related peptide (CGRP) cause vasodilation in epineurial arterioles of the sciatic nerve, which was impaired by diabetes. Treatment of diabetic rats (all groups) with enalapril or L-158809 completely prevented/reversed the diabetes-induced impairment in CGRP-mediated vascular relaxation. Treatment with enalapril or L-158809 was also effective in improving impaired acetylcholine-mediated vasodilation, but the efficacy was diminished from groups 1 to 4. These studies suggest that ACE inhibitors and/or angiotensin II receptor blockers may be effective treatments for diabetes and vascular and neural dysfunction. However, the efficacy of these treatments may be dependent on when the treatment is initiated. *Diabetes* 55:341–348, 2006

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CGRP, calcitonin gene–related peptide; MNCV, motor nerve conduction velocity.

ACE inhibition and/or blocking of the angiotensin II receptor are recognized as first-line treatment for hypertension as well as nephropathy and cardiovascular disease in diabetic patients (1–6). However, there is limited information available about the potential benefits of these drugs on diabetic neuropathy. In animal studies, Cameron and colleagues (7–10) have demonstrated that treating streptozotocin-induced diabetic rats with lisinopril or an angiotensin II receptor antagonist improved nerve function and modulated nerve blood flow. Aggarwal et al. (11) have also demonstrated that lisinopril treatment of diabetic rats prevented nerve dysfunction. However, these investigations were generally short-term intervention/prevention studies and failed to examine the potential antioxidant or neuroprotective role of these drugs in diabetic neuropathy. In studies of human diabetes, two small clinical trials demonstrated that diabetic neuropathy was improved by treatment of patients with trandolapril or lisinopril (12,13). However, as stated by the authors of one of these studies (12), more investigation is required before clinical practice can be advocated.

Most known effects of angiotensin II are mediated via activation of the AT_1-receptor. Activation of the AT_1-receptor is involved in vasoconstriction, inactivation of bradykinin, water and salt homeostasis, reactive oxygen species production, cellular hypertrophy and hyperplasia, and apoptosis (14). It has been shown that moderate hyperglycemia can increase plasma renin activity and mean arterial blood pressure in young male subjects with early uncomplicated diabetes (15). Angiotensin II has been demonstrated to stimulate NAD(P)H oxidase and increase oxidative stress in the kidney (16–18). NAD(P)H oxidases have been shown to be a primary source of reactive oxygen species generation in vascular tissue and a contributing factor in diabetic neuropathy (19,20). Furthermore, hyperglycemia and advanced glycation end products, two conditions associated with diabetes, have been shown to stimulate reactive oxygen species generation in vascular tissue via activation of NAD(P)H oxidase (21,22). However, in diabetes, activation of NAD(P)H oxidase may not be the only source for free radical production. Nishikawa et al. (23) have demonstrated that the mitochondria due to dysregulation of the electron transport chain is a major source of superoxide formation by cultured endothelial cells exposed to increased glucose concentration. Because of multiple potential sites for

superoxide formation in the vasculature of diabetic animals, it is important to determine the extent that ACE inhibitors prevent free radical production in different target tissues. For instance, we do not know how treatment with ACE inhibitors will affect oxidative stress in resistance vessels of diabetic rats.

To provide an answer as to whether blocking the renin-angiotensin system can prevent diabetic neuropathy, two issues must be addressed. First, does vascular dysfunction cause diabetic neuropathy, and, second, can ACE inhibitors or angiotensin II receptor antagonists ameliorate diabetic vascular dysfunction and hence neuropathy (24). Existing data suggest that increased oxidative stress and vascular dysfunction significantly contribute to the development and progression of diabetic neuropathy (23–30). It has also been demonstrated that treatment with ACE inhibitors improves endothelial dysfunction and reduces oxidative stress in diabetes (31–33). Therefore, it seems likely that ACE inhibitors will improve diabetic neuropathy. To address this issue, we performed studies with streptozotocin-induced diabetic rats treated with an ACE inhibitor or angiotensin II receptor antagonist. The treatment approach consisted of a prevention protocol and three intervention protocols with treatment being initiated after 4, 8, or 12 weeks of untreated diabetes. The treatment period was 12 weeks, and afterward vascular and neural function as well as oxidative stress was examined.

RESEARCH DESIGN AND METHODS

Unless stated otherwise, all chemicals used in these studies were obtained from Sigma Chemical (St. Louis, MO). Enalapril (L-proline, 1[N-[1-(ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]-(S)-,(Z)-2butenedioate) and L-158809 (5,7-dimethyl-2-ethyl-3-[[2′-[1H-tetrazol-5-yl][1,1′-biphenyl]-4-yl]methyl]-3H-imidazo [4,5-b]pyridine monohydrate) were kindly provided by Merck.

Male Sprague-Dawley (Harlan Sprague-Dawley, Indianapolis, IN) rats 13–14 weeks of age were housed in a certified animal care facility, and food (no. 7001; Harlan Teklad, Madison, WI) and water were provided ad libitum. All institutional and National Institutes of Health guidelines for use of animals were followed (ACURF 0212296). Diabetes was induced by intravenously injecting streptozotocin (55 mg/kg in 0.9% NaCl, adjusted to a pH 4.0 with 0.2 mol/l sodium citrate). Control rats were injected with vehicle alone. The rats were anesthetized with halothane before injection. Diabetes was verified 48 h later by evaluating blood glucose levels with the use of glucose-oxidase reagent strips (Lifescan, Milpitas, CA). Rats having a blood glucose level of ≥300 mg/dl (16.7 mmol/l) were considered to be diabetic. At this time, the diabetic rats were randomly divided into experimental groups. The experimental protocol consisted of four different periods for drug intervention. Group 1 was a prevention protocol, and treatment was initiated immediately after verification of diabetes. Groups 2, 3, and 4 were intervention protocols. For these groups, treatment was initiated after 4, 8, and 12 weeks of untreated diabetes, respectively. Each of these groups of rats contained a set of control rats, untreated diabetic rats, and diabetic rats treated with enalapril, an ACE inhibitor, or L-158809, an angiotensin receptor antagonist, or a combination of enalapril and L-158809. The drugs were added to the meal form of the diet, and the diet was pelleted for feeding purposes. For monotherapy, the dose of enalapril was 400 mg/kg diet. Based on diabetic rats' daily consumption, the amount of enalapril received on a daily basis by diabetic rats was 40 mg · kg^{-1} · rat^{-1}. This dose of enalapril totally blocked serum ACE activity. For monotherapy, the dose of L-158809 was 100–200 mg/kg diet. In initial studies, the dose of L-158809 that was used was 100 mg/kg diet. Results from this initial study demonstrated that the efficacy of L-158809 in improving diabetic neuropathy was less than we observed using enalapril. In the next study, the dose of L-158809 was increased to 200 mg/kg diet, but no difference in efficacy was observed. Therefore, results from the studies using L-158809 at 100 and 200 mg/kg diet were combined. Diabetic rats fed diet containing L-158809 received 10–20 mg · kg^{-1} · rat^{-1}. In some studies, diabetic rats were treated with a diet containing a combination of enalapril and L-158809. The dose of enalapril and L-158809 in the combination diet was 200 and 100 mg/kg diet, respectively. Control and untreated diabetic rats were fed nonsupplemented pelleted rat diet. The diet was made in the laboratory, dried in a vacuum oven, and stored refrigerated until use. Food consumption, body weight, and blood glucose were monitored weekly. Any diabetic rat that lost >10% of their initial body weight was treated with a low dose of insulin (2–3 units every other day) in order to maintain body weight. This amount of insulin treatment did not restore normoglycemia. Once initial body weight was restored, insulin treatment was stopped.

On the day of the experiment, blood glucose level was determined and the rats were intraperitoneally anesthetized with Nembutal (50 mg/kg i.p.; Abbott Laboratories, North Chicago, IL). Following the determination of motor nerve conduction velocity (MNCV) and endoneurial blood flow, the abdominal aorta was isolated and occluded 1–2 cm above the branch of the common iliac artery. The rats were then killed by exsanguination and body temperature lowered with topical ice.

MNCV. MNCV was determined as previously described using a noninvasive procedure in the sciatic-posterior tibial conducting system in a temperature-controlled environment (25–27). The MNCV was reported in meters per second.

Endoneurial blood flow. Immediately after determination of MNCV, sciatic nerve endoneurial nutritive blood flow was determined as previously described (25–27). The hydrogen clearance data were fitted to a mono- or biexponential curve using commercial software (Prism software; GraphPad, San Diego, CA) and nutritive blood flow (ml · min^{-1} · 100 g^{-1}), calculated and vascular conductance (ml · min^{-1} · 100 g^{-1} · mmHg^{-1}) determined by dividing nutritive blood flow by the average mean arterial blood pressure. Two recordings were made for each rat at different locations along the nerve, and the final blood flow value was averaged.

Vascular reactivity. Videomicroscopy was used to investigate in vitro vasodilatory responsiveness of arterioles vascularizing the region of the sciatic nerve (branches of the superior gluteal and internal pudendal arteries) and mesentery arteries as previously described (25–27). Cumulative concentration-response relationships were evaluated for acetylcholine (10^{-8}–10^{-4} mol/l) and calcitonin gene–related peptide (CGRP) (10^{-11}–10^{-8} mol/l) for epineurial arterioles and acetylcholine alone for mesentery arteries using vessels from control and treated and untreated diabetic rats. At the end of each dose-response determination, sodium nitroprusside (10^{-4} mol/l) was added to determine its vasodilation response. Afterward, we added papaverine (10^{-5} mol/l) to determine maximal vasodilation, which was consistently the same as the vascular tone of the resting vessel.

Detection of superoxide. Hydroethidine (Molecular Probes, Eugene, OR), an oxidative fluorescent dye, was used to evaluate in situ levels of superoxide (O_2^-) in epineurial vessels as described previously (25–27). Hydroethidine is permeable to cells and in the presence of O_2^- is oxidized to fluorescent ethidium bromide, where it is trapped by intercalating with DNA. This method provides sensitive detection of O_2^- in situ. Superoxide levels were also measured in the aorta by lucigenin-enhanced chemiluminescence as described previously (25–27).

Additional biological parameters. ACE activity in the serum was quantitated using a colorimetric assay kit from ALPCO diagnostics, and the data was presented as milliunits per milliliter serum. One unit of ACE activity is defined as the amount of enzyme required to release one micromole of hippuric acid per minute and per liter of serum at 37°C (Windham, NH).

Data analysis. The results are presented as means ± SE. Comparisons between the groups for MNCV, endoneurial blood flow, serum thiobarbituric acid–reactive substance, lens glutathione levels, and serum ACE activity were conducted using a one-way ANOVA and Newman-Keuls test for multiple comparisons (Prism software; GraphPad). Dose-response curves for acetylcholine- and CGRP-induced relaxation were compared using a two-way repeated-measures ANOVA with autoregressive covariance structure using the PROC MIXED program of SAS (25–27). Whenever significant interactions were noted, specific treatment dose effects were analyzed using a Bonferroni adjustment. A P value of <0.05 was considered significant.

RESULTS

Table 1 provides data on the final weight and nonfasting blood glucose level for the rats used in these studies from all four groups. The average starting weight for the rats used in this study was ~324 ± 6 g. Control rats in each of the four study groups gained weight. In contrast, diabetic rats lost ~10% of their body weight during the course of the study period. Treating diabetic rats with either enalapril or L-158809 did not influence this weight difference. All diabetic rats had an increased level of blood glucose that was not affected by either treatment. Table 1 also provides data on the level of superoxide in the aorta. Superoxide in the aorta was increased in untreated diabetic rats from all four groups compared with matched control rats. Treating

TABLE 1
Effect of diabetes and treatment with enalapril and L-158809 on body weight, blood glucose, and superoxide levels in the aorta

Conditions	n	Final weight (g)	Blood glucose (mg/dl)	Aorta superoxide (RLU)
Control (group 1)	8	469 ± 19	81 ± 3	2.39 ± 0.20
Diabetic (group 1)	8	291 ± 25*	495 ± 21*	5.87 ± 0.45*
Diabetic + enalapril (group 1)	9	292 ± 18*	461 ± 11*	2.86 ± 0.22†
Diabetic + L-158809 (group 1)	9	294 ± 11*	447 ± 20*	3.42 ± 0.46†
Control (group 2)	9	482 ± 7	67 ± 6	2.67 ± 0.21
Diabetic (group 2)	9	297 ± 15*	475 ± 14*	5.10 ± 0.38*
Diabetic + enalapril (group 2)	9	305 ± 17*	479 ± 31*	3.35 ± 0.27†
Diabetic + L-158809 (group 2)	10	304 ± 24*	481 ± 19*	3.28 ± 0.27†
Control (group 3)	8	524 ± 12	73 ± 2	2.88 ± 0.29
Diabetic (group 3)	9	293 ± 22*	417 ± 20*	5.14 ± 0.37*
Diabetic + enalapril (group 3)	8	298 ± 30*	494 ± 18*	3.14 ± 0.35†
Diabetic + L-158809 (group 3)	9	285 ± 15*	481 ± 18*	3.23 ± 0.40†
Control (group 4)	9	514 ± 11	73 ± 3	2.88 ± 0.22
Diabetic (group 4)	8	312 ± 14*	407 ± 22*	5.25 ± 0.31*
Diabetic + enalapril (group 4)	9	313 ± 23*	465 ± 23*	3.62 ± 0.38†
Diabetic + L-158809 (group 4)	10	306 ± 14*	471 ± 15*	4.34 ± 0.47*

Data are means ± SE. *$P < 0.05$ compared with control rats; †$P < 0.05$ compared with diabetic rats.

the diabetic rats with enalapril or L-158809 reduced levels of superoxide in the aorta in all four study groups compared with untreated diabetic rats, with the exception of L-158809 treatment in group 4. In group 4, diabetic rats untreated for 12 weeks, 12 weeks of treatment with L-158809 did not significantly reduce superoxide level in the aorta compared with untreated diabetic rats, and superoxide remained significantly elevated compared with controls. In this group, treatment with enalapril was also less effective in reducing superoxide levels in the aorta than it was in the other three groups. Treatment of diabetic rats with the combination of enalapril and L-158809 was equally to less effective in all four groups than monotherapy with enalapril in reducing superoxide level in the aorta of diabetic rats (data not shown). Efficacy studies for enalapril treatment of diabetic rats was assessed by determining serum ACE activity. Serum ACE activity in control, untreated diabetic, and enalapril-treated diabetic rats was 36.3 ± 4.0, 77.7 ± 18.7 ($P < 0.05$ compared with control), and 4.1 ± 1.1 ($P < 0.05$ compared with control and $P < 0.05$ compared with untreated diabetic rats, $n = 15$ rats with serum samples being used from rats of each of the four study groups) mU/ml serum, respectively.

Data in Table 2 show the effect enalapril and L-158809 treatment of diabetic rats has on endoneurial blood flow in the sciatic nerve and MNCV. Diabetes caused a decrease in endoneurial blood flow and MNCV in all four groups of rats. Treating diabetic rats with enalapril prevented/improved the diabetes-induced decrease in endoneurial blood flow and MNCV. Treatment of diabetic rats with L-158809 also prevented/improved the diabetes-induced decrease in endoneurial blood flow and MNCV but was generally less effective than enalapril treatment. Combination therapy consisting of enalapril and L-158809 was only as effective as enalapril alone (data not shown).

Previously, we demonstrated that vascular relaxation of

TABLE 2
Effect of diabetes and treatment with enalapril and L-158809 on endoneurial blood flow and MNCV

Conditions	n	Endoneurial blood flow nutritive ($ml \cdot min^{-1} \cdot 100\ g^{-1}$)	Endoneurial blood flow conductance ($ml \cdot min^{-1} \cdot 100\ g^{-1} \cdot mmHg^{-1}$)	MNCV (m/s)
Control (group 1)	8	23.0 ± 3.5	0.164 ± 0.030	60.2 ± 2.5
Diabetic (group 1)	8	10.7 ± 1.3*	0.073 ± 0.008*	40.8 ± 1.5*
Diabetic + enalapril (group 1)	9	18.8 ± 1.3†	0.152 ± 0.012†	54.1 ± 2.5†
Diabetic + L-158809 (group 1)	9	24.4 ± 4.5†	0.182 ± 0.032†	49.4 ± 0.8*†
Control (group 2)	9	24.9 ± 3.3	0.184 ± 0.026	62.8 ± 2.2
Diabetic (group 2)	9	9.6 ± 1.1*	0.067 ± 0.009*	40.1 ± 1.9*
Diabetic + enalapril (group 2)	9	18.8 ± 2.2†	0.161 ± 0.022†	55.4 ± 1.3†
Diabetic + L-158809 (group 2)	10	13.4 ± 2.8	0.108 ± 0.022	51.5 ± 2.2*†
Control (group 3)	8	20.4 ± 3.5	0.157 ± 0.025	56.2 ± 1.4
Diabetic (group 3)	9	8.0 ± 3.1*	0.064 ± 0.018*	40.9 ± 1.4*
Diabetic + enalapril (group 3)	8	21.4 ± 2.9†	0.174 ± 0.030†	51.3 ± 3.0†
Diabetic + L-158809 (group 3)	9	19.4 ± 4.8	0.152 ± 0.038	46.2 ± 2.3*
Control (group 4)	9	24.7 ± 2.9	0.180 ± 0.020	58.1 ± 3.0
Diabetic (group 4)	8	10.3 ± 2.6*	0.073 ± 0.019*	41.9 ± 1.4*
Diabetic + enalapril (group 4)	9	22.6 ± 3.5†	0.167 ± 0.025†	59.3 ± 3.4†
Diabetic + L-158809 (group 4)	10	17.5 ± 2.6	0.132 ± 0.021	50.5 ± 2.4†

Data are means ± SE. *$P < 0.05$ compared with control rats; †$P < 0.05$ compared with diabetic rats.

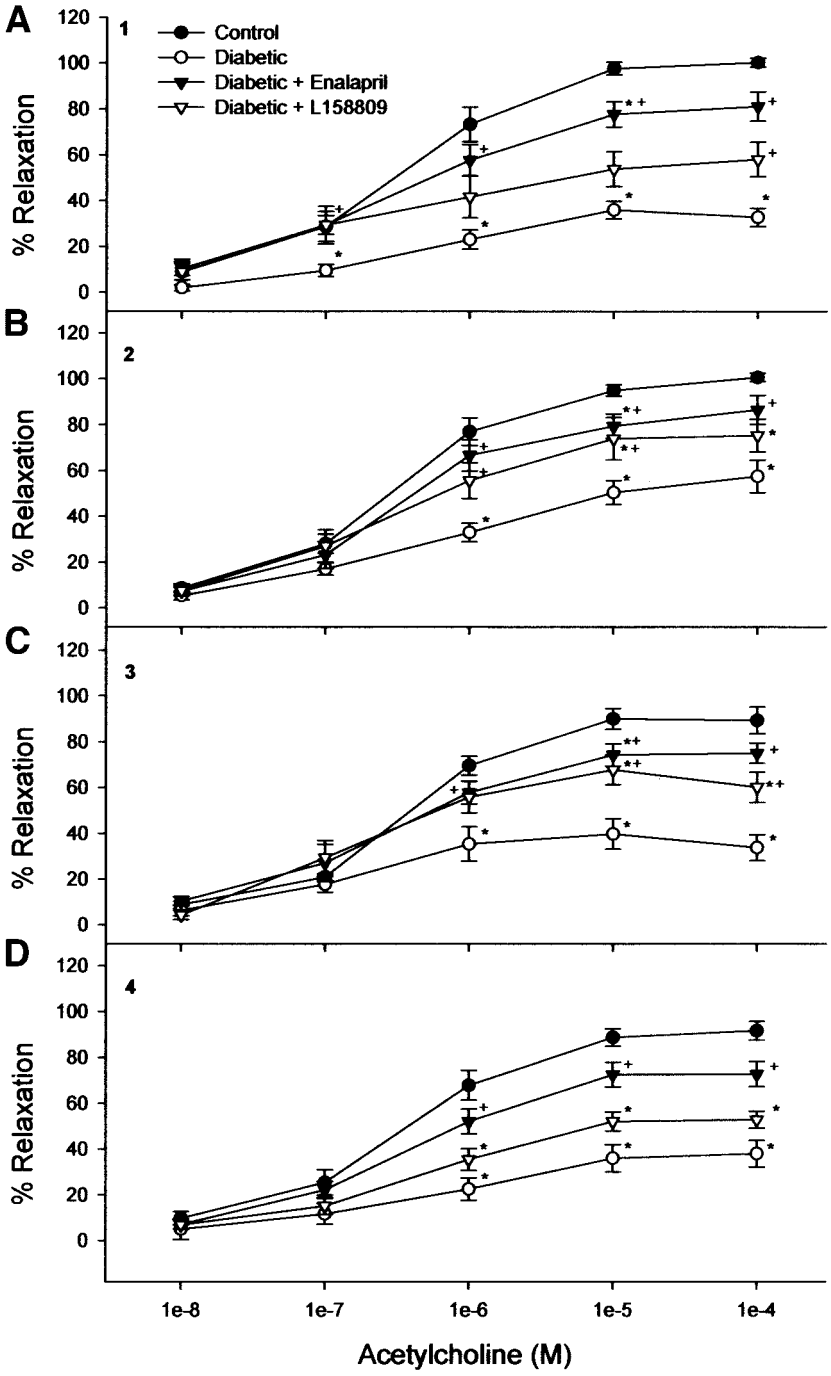

FIG. 1. Determination of acetylcholine-mediated vascular relaxation in epineurial arterioles of the sciatic nerve from control rats, diabetic rats, and diabetic rats treated for 12 weeks with enalapril or L-158809 2 days after injection with streptozotocin (group 1) or following 4, 8, and 12 weeks of untreated diabetes (groups 2, 3, and 4, respectively). Pressurized arterioles (40 mmHg) were constricted with U46619 (30–50%), and incremental doses of acetylcholine were added to the bathing solution while recording steady-state vessel diameter. Data are presented as the mean of percent relaxation ± SE. The number of experimental determinations for control, untreated diabetic, enalapril-treated diabetic, and L-158809–treated diabetic rats was for groups 1 (11, 9, 13, and 11), 2 (10, 12, 11, and 15), 3 (9, 9, 8, and 10), and 4 (11, 9, 15, and 16). *P < 0.05 compared with age-matched control rats; †P < 0.05 compared with untreated diabetic rats.

epineurial arterioles of the sciatic nerve to acetylcholine and CGRP are impaired by diabetes (34,35). In this study, we examined whether treating diabetic rats with enalapril or L-158809 for 12 weeks using a prevention protocol or for 12 weeks following 4, 8, or 12 weeks of untreated diabetes can prevent vascular dysfunction. Figure 1 presents data for acetylcholine. Acetylcholine-mediated vascular relaxation was maximally impaired after 12 weeks of diabetes. In all four study groups, treatment of diabetic rats with enalapril was more effective in preventing/reversing the decrease in vascular relaxation in response to acetylcholine than was treatment with L-158809. In study group 4, treatment of diabetic rats for 12 weeks with L-158809, after 12 weeks of untreated diabetes, did not significantly reverse the diabetes-induced decrease in vascular relaxation to acetylcholine, whereas treatment with enalapril was effective. Treatment with the combination of enalapril and L-158809 were generally as effective as enalapril alone (data not shown). Relaxation of epineurial arterioles to 10^{-4} mol/l sodium nitroprusside (maximal concentration) was impaired 25–40% by diabetes. Treatment of diabetic rats with enalapril completely prevented/reversed this deficit and was more effective than treatment of diabetic rats with L-158809. Data in Fig. 2 demonstrate that treating diabetic rats with enalapril or L-158809 for 12 weeks (Fig. 2*A*, group 1) or for 12 weeks following 12 weeks of untreated diabetes (Fig. 2*B*, group 4) completely prevented the diabetes-induced decrease in relaxation in response to CGRP. Treatment of diabetic rats with the combination of enalapril and L-158809 was also similarly effective (data not shown).

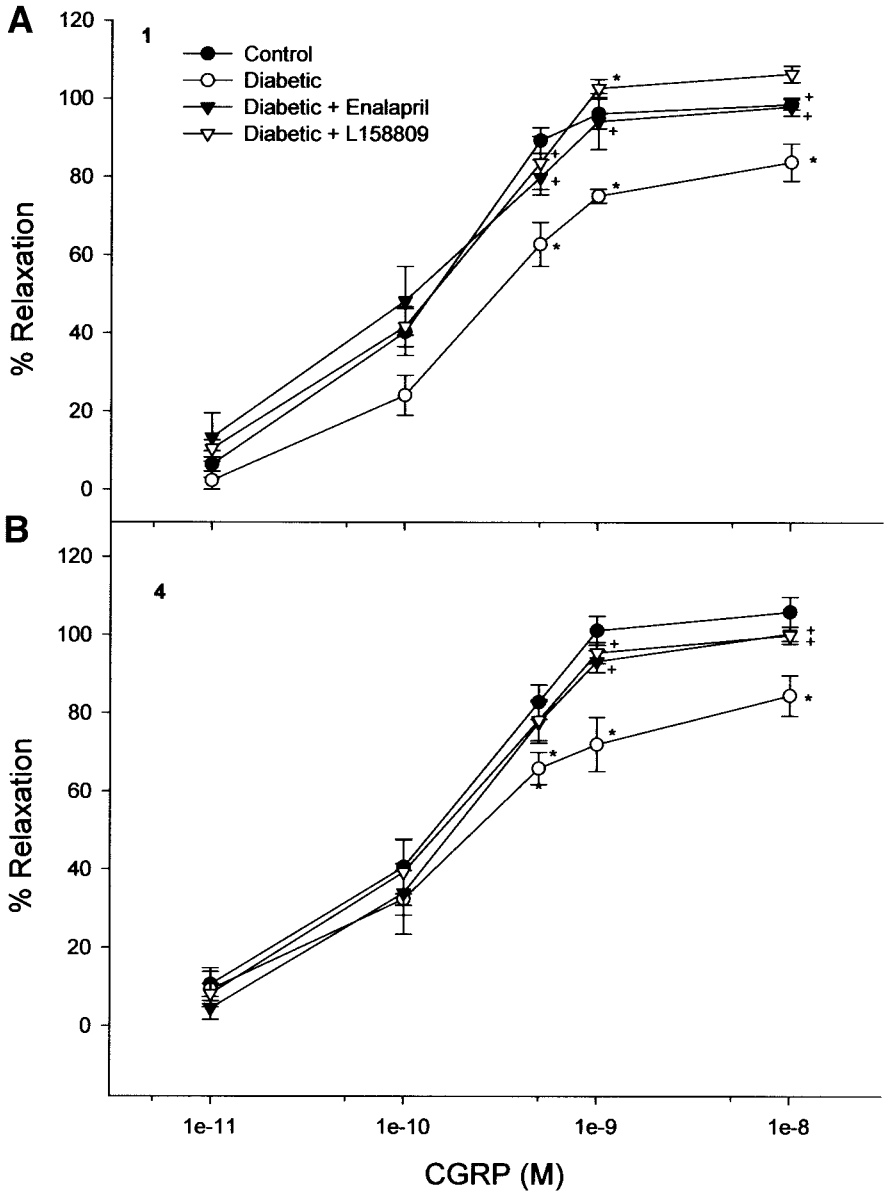

FIG. 2. Determination of CGRP-mediated vascular relaxation in epineurial arterioles of the sciatic nerve from control rats, diabetic rats, and diabetic rats treated for 12 weeks with enalapril or L-158809 2 days after injection with streptozotocin (group 1) or following 12 weeks of untreated diabetes (group 4). Pressurized arterioles (40 mmHg) were constricted with U46619 (30–50%), and incremental doses of CGRP were added to the bathing solution while recording steady-state vessel diameter. Data are presented as the mean of percent relaxation ± SE. The number of experimental determinations for control, untreated diabetic, enalapril-treated diabetic, and L-158809–treated diabetic rats was for group 1 (11, 9, 13, and 11) and group 4 (11, 9, 15, and 16). *$P < 0.05$ compared with age-matched control rats; †$P < 0.05$ compared with untreated diabetic rats.

We also performed an abbreviated study of the effect of diabetes and treatment with enalapril on vascular reactivity using mesenteric arteries from group 3 rats (rats treated for 12 weeks with enalapril following 8 weeks of untreated diabetes). These studies were performed to determine whether the beneficial affects of enalapril treatment on acetylcholine-mediated vascular relaxation in epineurial arterioles also occurs in another vascular bed of resistance-size vessels. Data in Fig. 3 demonstrate that vascular relaxation to acetylcholine in mesenteric arteries was impaired by diabetes and that treating diabetic rats with enalapril improved the responsiveness of these vessels to acetylcholine.

Data in Fig. 4 demonstrate that superoxide level was increased in epineurial arterioles of the sciatic nerve of diabetic rats from groups 1 and 3. Treating diabetic rats with enalapril reduced superoxide levels in epineurial arterioles by ~50%. In contrast, it did not appear that treatment of diabetic rats with L-158809 reduced superoxide levels.

DISCUSSION

Hyperglycemia increases tissue angiotensin II, which induces oxidative stress, endothelial damage, and disease pathology including vasoconstriction, thrombosis, inflammation, and vascular remodeling (14,36). Blocking the tissue rennin-angiotensin system has been shown to improve diabetic nephropathy, but little is known whether ACE inhibitors or angiotensin II receptor antagonists can prevent diabetic neuropathy (1–3,6–13). In these studies, we examined whether treatment of streptozotocin-induced diabetic rats with enalapril or L-158809 as monotherapy or in combination, using prevention and intervention protocols, could prevent/reverse diabetes-induced vascular and neural dysfunction. The major findings from this study were first that treating diabetic rats with the ACE inhibitor enalapril or the angiotensin II receptor antagonist L-158809 improved diabetes-induced vascular and neural dysfunction. Significant improvement was observed even when treatment was not initiated until after 12 weeks of untreated diabetes. Second, generally we found that treatment

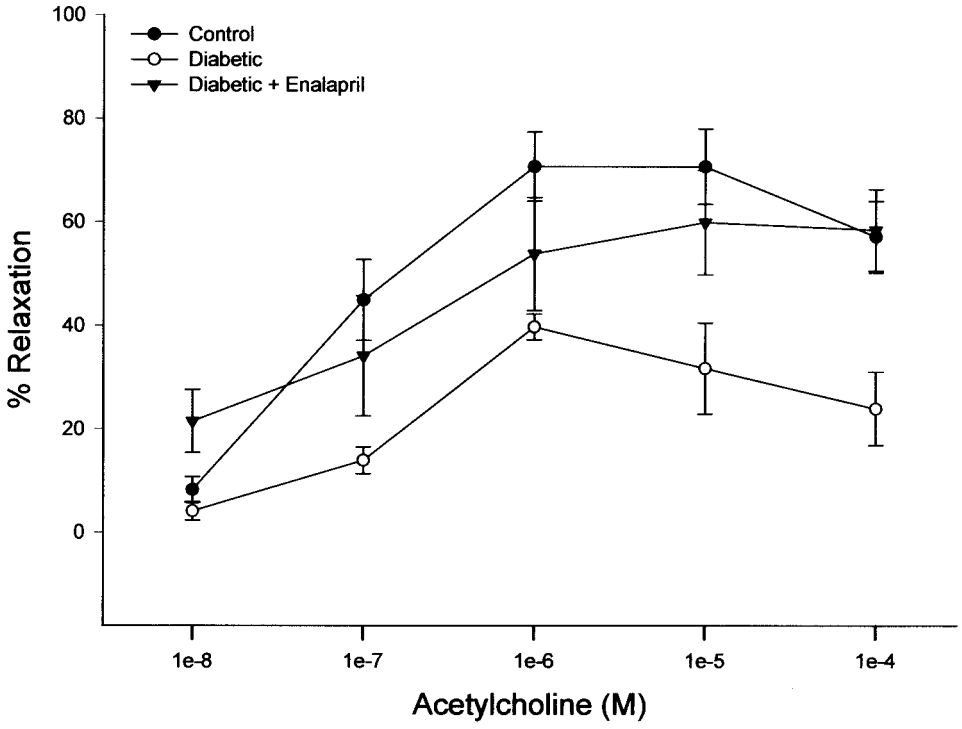

FIG. 3. Determination of acetylcholine-mediated vascular relaxation in mesenteric arteries from control rats, diabetic rats, and diabetic rats treated for 12 weeks with enalapril following 8 weeks of untreated diabetes (groups 3). Pressurized arterioles (40 mmHg) were constricted with U46619 (30–50%), and incremental doses of acetylcholine were added to the bathing solution while recording steady-state vessel diameter. Data are presented as the mean of percent relaxation ± SE. The number of experimental determinations for control, untreated diabetic, and enalapril-treated diabetic rats was 11, 9, and 7, respectively. *P < 0.05 compared with age-matched control rats.

with enalapril was more effective in preventing/reversing diabetes-induced vascular and neural dysfunction than treatment with the angiotensin II receptor antagonist L-158809. Unlike some reports suggesting synergy for treatment of diabetic nephropathy or hypertension when ACE inhibitors were combined with angiotensin II receptor antagonists, we found no evidence of synergy for preventing/reversing vascular and neural dysfunction related to diabetic neuropathy when we combined enalapril and L-158809 (37–39). Third, we found that treatment of diabetic rats with enalapril or L-158809 prevented/reversed superoxide formation by the aorta. However, enalapril treatment was only partially effective and treatment with L-158809 was noneffective in preventing/reversing superoxide formation in epineurial arterioles of the sciatic nerve. This is consistent with previous studies that have demonstrated that these drugs can prevent angiotensin II stimulation of NAD(P)H oxidase activity in large vessels associated with hypertension or diabetes and subsequent increased formation of superoxide (15,16,31,40–42). Our previous studies have suggested that the mitochondria may be the primary contributor to superoxide formation by epineurial arterioles derived from diabetic rats (43). Therefore, it appears that enalapril and to a greater extent L-158809 may have limited capability of preventing superoxide formation and oxidative stress in resistance-size vessels derived from diabetic rats. This difference in the ability of enalapril and L-158809 to prevent formation of superoxide in resistance vessels may be one reason for the general better efficacy of enalapril in correcting diabetic vascular dysfunction.

Previously, we demonstrated that treating streptozotocin-induced diabetic rats with antioxidants significantly improved diabetic vascular and neural dysfunction (25,26). In these studies, we found that enalapril and to a lesser extent L-158809 were effective in preventing as well as reversing diabetes-induced vascular and neural dysfunction. This suggests that these drugs, in addition to improving oxidative stress, may also provide other beneficial actions that contribute to improving diabetes-induced impairment in vascular and nerve function.

Cameron and colleagues (7,8) previously demonstrated that treatment of streptozotocin-induced diabetic rats with an ACE inhibitor or an angiotensin receptor antagonist improved nerve function and nerve blood flow and stimu-

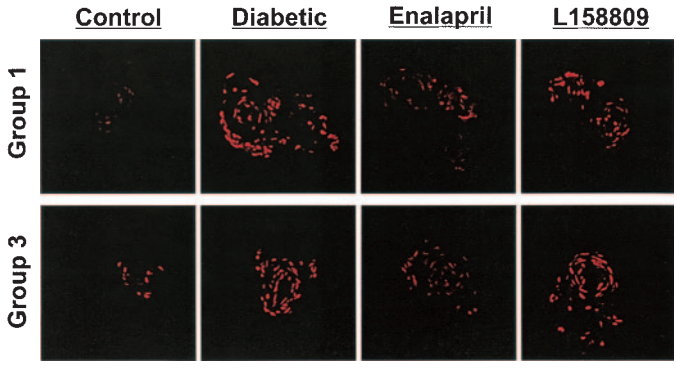

FIG. 4. Detection of superoxide in epineurial arterioles of the sciatic nerve from control rats, diabetic rats, and diabetic rats treated for 12 weeks with enalapril or L-158809 2 days after injection with streptozotocin (group 1) or following 8 weeks of untreated diabetes (group 3). Presented are fluorescent photomicrographs of confocal microscopic sections of epineurial arterioles of the sciatic nerve. Each of the four vessels was examined on the same day. Arterioles were labeled with the oxidative dye hydroethidine, as described in the RESEARCH DESIGN AND METHODS section. Recording of fluorescence was taken at identical laser and photomultiplier settings for each vessel cross-section. Analysis of these images using Carl Zeiss LSM Image Examiner software indicated that expression of superoxide compared with control was 2.8 ± 0.4*, 1.4 ± 0.2†, and 2.0 ± 0.4 (group 1) and 2.2 ± 0.3*, 1.3 ± 0.1 †, and 1.9 ± 0.3 (group 3) for untreated diabetic rats, enalapril-treated diabetic rats, and L-158809–treated diabetic rats, respectively (*P < 0.05 compared with control; †P < 0.05 compared with untreated diabetic rat). For these analyses, the superoxide value for control rats was arbitrarily assigned a value of 1. These values were obtained from three rats per group and treatment condition, and two vessel segments were analyzed for each individual rat. Shown is a representative sample from multiple sections from each evaluation.

lated angiogenesis. Our studies also demonstrated that treating diabetic rats with an ACE inhibitor or angiotensin receptor antagonist improved nerve function and endoneurial blood flow. Moreover, our studies demonstrated that diabetes-induced impairment of vascular relaxation by epineurial arterioles of the sciatic nerve in response to acetylcholine and CGRP was significantly improved. Unlike previous studies, we performed both prevention and intervention protocols with the most extreme condition being treatment initiation delayed for 12 weeks. Even in this study group, we found that enalapril and to a lesser extent L-158809 were capable of improving diabetic vascular and neural dysfunction.

Previously, we have shown that diabetes caused impairment in vascular relaxation in response to acetylcholine and CGRP in epineurial arterioles of the sciatic nerve (34,35). Acetylcholine-induced vascular relaxation is endothelium dependent and mediated by nitric oxide and endothelium-derived hyperpolarizing factor, and both are impaired by diabetes (34,43,44). Moreover, impairment by diabetes of acetylcholine-mediated vascular relaxation of epineurial arterioles precedes slowing of MNCV, suggesting that vascular dysfunction contributes to impaired nerve activity (27). CGRP-mediated vascular relaxation is endothelium independent, and impairment by diabetes requires 10–12 weeks, unlike acetylcholine-mediated relaxation which is impaired after 1 week of diabetes (34,35). These studies have demonstrated that enalapril and to a lesser extent L-158809 treatment is capable of preventing/reversing the diabetes-induced impairment of relaxation by acetylcholine and CGRP. The improvement in vascular relaxation to acetylcholine and CGRP was independent of a correction of superoxide formation by epineurial arterioles of streptozotocin-treated diabetic rats. In epineurial arterioles of the sciatic nerve, we demonstrated that treatment of diabetic rats with enalapril reduced superoxide formation by ~50–75%, and with L-158809 treatment the decrease in superoxide level in epineurial arterioles was minimal. In the aorta, treatment with enalapril or L-158809 almost completely prevented superoxide formation. Likewise, when streptozotocin-induced diabetic rats were treated with α-lipoic acid, the increase in superoxide formation in epineurial arterioles was completely prevented (25). Improvement of acetylcholine-mediated vascular relaxation may be due to increased formation of nitric oxide capable of overcoming quenching by superoxide and/or improved formation/activity of endothelium-derived hyperpolarizing factor (33,45,46). In this regard, Kihara et al. (47) have demonstrated that treatment of diabetic rats with ACE inhibitors improve diabetic neuropathy by increasing nitric oxide synthase synthesis.

Our studies demonstrated that treatment of diabetic rats with enalapril or L-158809 using a prevention protocol or after 12 weeks of untreated diabetes, prevented the diabetes-induced impairment in vascular relaxation mediated by CGRP. Interestingly, Kawasaki and colleagues (48,49), in studies with the mesenteric artery, have demonstrated an age-related decrease in CGRP-mediated vasodilation, neurogenic CGRP release, and CGRP mRNA levels in the dorsal root ganglion in spontaneously hypertensive rats, indicating a reduced function of CGRPergic nerves. They found that treatment of these rats with ACE inhibitor or angiotensin II receptor antagonist restored the reduced function of CGRP. In another rat hypertensive model, treating hypertensive rats with an ACE inhibitor or angiotensin II receptor antagonist increased plasma levels of CGRP and expression of CGRP mRNA in dorsal root ganglia (50). In our studies, treatment of diabetic rats with enalapril or L-158809 completely restored reactivity to CGRP in epineurial arterioles. It is possible that treatment of diabetic rats with an ACE inhibitor or angiotensin II receptor antagonist was increasing CGRP expression and restoring bioactivity in epineurial arterioles as reported in mesenteric arteries, which are also resistance-size vessels. Further studies will be required to confirm this explanation.

In summary, these studies demonstrate that treatment of diabetic rats with an ACE inhibitor and to a lesser degree an angiotensin II receptor antagonist provides an effective approach to preventing/reversing diabetes-induced vascular and neural dysfunction. It appears that the mechanism responsible for the beneficial affects of treatment by these drugs on diabetic neuropathy is not solely due to improving oxidative stress and that additional mechanisms including nerve protection, perhaps of dorsal root ganglion neurons, must also contribute to the affect of these drugs on diabetic neuropathy.

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