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Original Contribution

EFFECT OF α-LIPOIC ACID ON VASCULAR RESPONSES AND NOCICEPTION IN DIABETIC RATS

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Abstract—Oxidative stress contributes to the vascular and neurological complications of diabetes mellitus. The aim was to evaluate the effects of treatment with the radical scavenger and transition metal chelator, α-lipoic acid, on endothelium-dependent relaxation of the mesenteric vasculature and on superior cervical ganglion blood flow in 8 week streptozotocin-induced diabetic rats. α-Lipoic acid effects on small nerve fiber-mediated nociception were also monitored. For the in vitro phenylephrine-precontracted mesenteric vascular bed, diabetes caused a 31% deficit in maximum endothelium-dependent relaxation to acetylcholine, and a 4-fold reduction in sensitivity. α-Lipoic acid gave 85% protection against these defects. Acetylcholine responses are mediated by nitric oxide and endothelium-derived hyperpolarizing factor: isolation of the latter by nitric oxide synthase blockade revealed a 74% diabetic deficit that was halved by α-lipoic acid. Superior cervical ganglion blood flow, 52% reduced by diabetes, was dose-dependently restored by α-lipoic acid (ED_{50}, 44 mg/kg/d). Diabetic rats exhibited mechanical and thermal hyperalgesia, which were abolished by α-lipoic acid treatment. Thus, diabetes impairs nitric oxide and endothelium-derived hyperpolarizing factor-mediated vasodilation. This contributes to reduced neural perfusion, and may be responsible for altered nociceptive function. The effect of α-lipoic acid strongly implicates oxidative stress in these events and suggests a potential therapeutic approach. © 2001 Elsevier Science Inc.

Keywords—Diabetes mellitus, Vascular endothelium, Nitric oxide, EDHF, Oxidative stress, Mesenteric vasculature, Autonomic nervous system, Blood flow, Pain, Neuropathy, Free radicals

INTRODUCTION

Diabetes mellitus causes vascular complications such as nephropathy, retinopathy, and neuropathy, which increase mortality and reduce the quality of life [1,2]. Vascular endothelium appears to be particularly vulnerable. Nitric oxide (NO)-mediated endothelium-dependent relaxation is impaired in animal models of diabetes, for vessels and vascular beds including aorta [3–6], basilar artery [7], corpus cavernosum [8], vasa nervorum [9,10], kidney [11], heart [12], and mesenteric bed [13]. Similar deficits have been noted in several studies on patients [14,15].

Diabetes also impairs vasodilation by mediators other than NO, including prostanoids and endothelium-derived hyperpolarizing factor (EDHF) [1]. The mesenteric vasculature provides a useful preparation in which to study EDHF because a substantial component of endothelium-dependent relaxation to agonists such as acetylcholine (ACh) remains after the NO and prostanoid systems are blocked [16–18]. Streptozotocin-induced diabetes markedly impairs EDHF-mediated smooth muscle hyperpolarization and vasodilation in rat mesenteric resistance vessels [13]. The identity of EDHF is not established, however, it promotes K^+ efflux through small conductance Ca^{2+}-dependent channels [17,18]. EDHF is particularly important in arterioles controlling tissue nutritive perfusion [16–18] and could, therefore, have special relevance for microvascular complications.

One cause of the diabetic endothelial deficit is the elevated level of reactive oxygen species (ROS), which react with NO to neutralize vasodilator activity [19]. Defects in endothelium-dependent relaxation of large and small vessels may be prevented by antioxidant treatment in diabetic rats [12,20–22], although negative find-

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ings for mesenteric vessels were noted [23]. α-Lipoic acid (LA) is a powerful antioxidant, acting as a scavenger and metal chelator [24], which improved sciatic nerve perfusion, glutathione content, and conduction velocity, and corpus cavernosum endothelium-dependent and neurogenic relaxation in diabetic rats [25–27]. It is not known whether antioxidants affect the diabetic EDHF deficit. Therefore, one aim was to assess the effects of preventive LA treatment on endothelial function using the in vitro rat mesenteric vascular bed preparation.

While LA effects on nerve blood flow and large-diameter motor and sensory nerve fiber function have been documented [25,26], there is no information about potential actions on blood flow to the ganglia that house the cell bodies of origin for autonomic and sensory fibers. Thus, a second aim was to assess LA-treatment effects on superior cervical ganglion blood flow, which is reduced in long-term diabetes [28]. The third aim was to assess function in small somatic fiber systems that underlie nociception, as painful neuropathy is an important neurological complication of diabetes [2].

MATERIALS AND METHODS

The experiments were performed in accordance with regulations specified by the United Kingdom "Animal Procedures Act, 1986," and the National Institutes of Health "Principles of Laboratory Animal Care, 1985 revised version."

Experimental groups and diabetes induction

Male Sprague Dawley rats (Aberdeen University colony) were used, aged 19 weeks at the start of experiments. Diabetes was induced by intraperitoneal injection of streptozotocin (Astra-Zeneca, Macclesfield, Cheshire, UK) freshly made up in sterile saline solution, at a dose of 40–45 mg/kg. Diabetes was verified after 24 h by the presence of hyperglycemia and glucosuria (Visidex II and Diastix; Ames, Slough, UK) in nonfasted rats. In final experiments, plasma glucose was estimated (GOD-Perid method; Boehringer Mannheim, Mannheim, Germany) on samples taken from the tail vein or carotid artery. Diabetes duration was 8 weeks. Two investigations were undertaken. In *study 1*, the aim was to examine responses of the mesenteric vascular bed. The study was preventive, one group acted as diabetic controls and another group was treated with LA as a dietary supplement (dose approximately 300 mg/kg/d racemate; ASTA Medica AWD, Frankfurt, Germany) from diabetes induction. Untreated nondiabetic controls were used and a group of nondiabetic rats were given 300 mg/kg/d LA treatment for 8 weeks. Dietary admixture was chosen for LA delivery, as opposed to intraperitoneal injection, to avoid a potential disruptive effect of daily injection on the mesenteric vascular. Previous studies have shown that this dose/route combination was effective against neural and vascular dysfunction of corpus cavernosum in diabetic rats [27]. In *study 2*, the aims were to examine superior cervical ganglion (SCG) blood flow and nociceptive responses. This was an intervention study, with LA treatment being given for the last 2 weeks of the 8 week period by daily intraperitoneal injection, at doses of 20, 50, and 100 mg/kg. For intraperitoneal injection, LA powder was mixed with sterile saline and NaOH was added until the suspension dissolved. The pH was then brought to 7.4 with HCl. SCG blood flow was measured for all LA doses; nociception thresholds were only determined in the 100 mg/kg group, rats being tested before and after the treatment period.

Mesenteric vascular preparation

In final experiments, rats were anesthetized (4% halothane in air), the superior mesenteric artery was cannulated, and the mesenteric vascular bed was cut away close to the intestine. The mesenteric bed was prepared as previously described [29], mounted on a glass surface at 37°C, with free drainage, and was covered with plastic film. A peristaltic pump was used to intraluminally perfuse the preparation at a constant rate (5 ml/min) with modified Krebs-Ringers solution (144.0 Na^+, 5.0 K^+, 1.25 Ca^{2+}, 1.1 Mg^{2+}, 25.0 HCO_3^-, 1.1 PO_4^{3-}, 1.1 SO_4^{2-}, 5.5 glucose; in mM) at 37°C, which was gassed continuously with 95% O_2 : 5% CO_2 (pH 7.35). A transducer connected close to the mesenteric artery cannulation point monitored perfusion pressure, and drug responses were registered as pressure changes. The peristaltic pump was set up so that three inputs were available for the delivery of Krebs-Ringer and/or drugs dissolved in Krebs-Ringer solution.

The mesenteric bed was equilibrated for 60 min, then constricted with phenylephrine (100 μM), allowed to relax for 30 min, and then preconstricted with phenylephrine (3–100 μM). Cumulative concentration-response relationships were determined for endothelium-dependent relaxation to ACh. After recovery, in some preparations, pressor concentration-response curves to phenylephrine were recorded followed by relaxation, and redetermination of the phenylephrine concentration-response relationship in the presence of 1–10 mM of the NO synthase inhibitor, N^G-nitro-L-arginine (L-NNA). ACh concentration-responses were also determined in the presence of L-NNA, to isolate the EDHF component. This was unaffected by addition of 5 μM of the cyclooxygenase inhibitor, flurbiprofen, to the perfusate, but was completely abolished by increasing K^+ to 40–60

mM. Finally, again in the presence of L-NNA and phenylephrine precontraction, cumulative concentration-response curves were constructed for the NO donor, sodium nitroprusside.

Superior cervical ganglion blood flow

Rats were anesthetized with thiobutabarbital (Astra-Zeneca; 50–100 mg/kg), by intraperitoneal injection. The trachea was cannulated for artificial ventilation. A cannula in the right carotid artery was used to monitor mean systemic blood pressure. Core temperature of the rat was monitored and regulated at 37–38°C, using a rectal probe and radiant heat. The left SCG was located in the vicinity of the carotid bifurcation. Skin around the neck incision was used to form a pool that was filled with mineral oil maintained at 35–37°C by radiant heat during measurements. SCG blood flow was estimated by microelectrode polarography and hydrogen clearance by a slightly modified method to that previously described for sciatic nerve [30]. Briefly, a glass-insulated platinum microelectrode was inserted into the SCG and polarized at 0.24 V with respect to a subcutaneous reference electrode. Ten percent H_2 was added to the inspired gas, the proportions of O_2 and N_2 being adjusted to 20% and 70%, respectively. When the H_2 current recorded by the electrode had stabilized, indicating equilibrium with arterial blood, the H_2 supply was shut off and N_2 delivery was increased appropriately. The H_2 clearance curve was recorded until baseline, the latter being defined as no systematic decline in electrode current over 1 min. This procedure was then repeated at another SCG site. After the experiment, clearance curves were digitized and monoexponential or biexponential curves were fitted to the data by computer using nonlinear regression software that employed the Marquardt algorithm and the least squares method for optimizing goodness-of-fit (Prism, Graphpad, San Diego, CA, USA). The slow exponent was taken to reflect nutritive flow [31].

Small fiber sensory responses

Nociceptive thresholds for mechanical stimulation were measured by the Randall-Sellito test [32] and latencies for withdrawal reflexes to noxious thermal stimulation of the foot were estimated by the Hargreaves plantar test [33] using commercially available equipment (Ugo-Basile, Comerio, Italy). Briefly, tests were carried out in a constant temperature room at the same time each day, and rats were given a 3 d period for familiarization with handling, the environment and equipment, and the measurement procedure. Mechanical pressure thresholds were then estimated twice per day for each foot over a 3 d period before LA treatment commenced. After 12 d of treatment, thresholds were again determined over 3 d. Data from the 3 pretreatment and 3 end-of-treatment days were averaged to give pressure threshold values. Each day following mechanical testing, rats were placed in the thermal testing apparatus, which consisted of a Plexiglas enclosure with a glass base, in which they were free to move. After 30 min acclimatization, a constant power infrared stimulus was focused through the glass base onto the sole of the foot and the latency for reflex foot withdrawal automatically recorded via a photoelectric monitor. For each session, four measurements were obtained, two from each foot, the average being taken as the final withdrawal latency. As with the mechanical estimates, there was a 3 d run-in period, followed by 3 d predrug and 3 d end-of-drug testing.

Table 1. Non-fasted Plasma Glucose Concentrations and Body Weights for the Groups of Rats Used in the Studies

Group	*n*	Plasma glucose (mM)	Body weight (g)
Nondiabetic	22	8.0 ± 0.4	433 ± 3
Nondiabetic + lipoic acid	12	11.8 ± 1.2	409 ± 4
Diabetic	20	39.0 ± 1.7*	335 ± 8*
Diabetic + lipoic acid	41	40.5 ± 1.1*	341 ± 5*

Data are mean ± SEM; * $p < .001$ vs. nondiabetic groups.

Statistical analysis

Results are expressed as means ± SEM. Data were subjected to Bartlett's test for homogeneity of variances, followed by log transformation if necessary before one-way analysis of variance. Where significance was reached ($p < .05$), between-group differences were established using the Student-Neuman-Keuls multiple comparison test. If variances were not homogenous, data were analyzed by an appropriate nonparametric test, such as Kruskal-Wallis' one-way analysis of variance followed by Dunn's multiple comparison test. Within-group serial comparisons (ACh or phenylephrine effects before and after L-NNA, or nociception responses before and after LA treatment) were made using paired Student's *t*-tests. Concentration-response data were fitted by sigmoid curves using the least squares method to estimate EC_{50} (Prism, Graphpad).

RESULTS

Diabetes rats had body weights approximately 22% less than those of the nondiabetic groups and nonfasted plasma glucose concentration was elevated approximately 4.9-fold (Table 1). These parameters were not affected by LA treatment.

Study 1: mesenteric vascular bed

Concentration-response curves for endothelium-dependent relaxation to ACh in the phenylephrine-precontracted mesenteric vascular bed are shown in Fig. 1A. Diabetes caused a 30.8 ± 5.0% reduction ($p < .001$) in maximum relaxation and a rightward shift of the dose response curve. Responses to ACh doses of 10 nM and above were depressed by diabetes ($p < .05$). The diabetic change in maximum relaxation was 89.0 ± 6.0% prevented ($p < .01$) by LA treatment. ACh responses did not differ significantly from those of nondiabetic rats and were greater than those of the diabetic group at doses of 3 nM and greater ($p < .05$). In nondiabetic rats, LA treatment did not significantly alter maximum relaxation.

High-dose L-NNA pre- and co-perfusion depressed ACh responses ($p < .001$) in all groups (Fig. 1B). In nondiabetic rats, maximum relaxation was reduced from approximately 98 to 60%, irrespective of LA treatment. The largest effect of L-NNA was noted for the diabetic group, with a 73.8 ± 6.5% reduction in maximum relaxation. In 2 out of 12 diabetic rats, no response to ACh was detected in the presence of L-NNA. Compared to the nondiabetic group, responses to ACh were diminished at doses of 10 nM and above. LA treatment in nondiabetic rats did not affect ACh responses, however, the diabetic deficit was attenuated ($p < .05$) at doses of 0.3 μM and greater. The protective effect of LA was 40.6 ± 14.2% ($p < .05$) against impaired maximum relaxation. Coperfusion with the cyclooxygenase inhibitor, flurbiprofen, (5 μM for 30 min) did not significantly alter responses to ACh in the presence of L-NNA, which is shown (Fig. 1C) for subgroups of untreated and LA-treated diabetic rats.

The shifts to the right for ACh concentration-response curves with diabetes and L-NNA were reflected in (-log)EC_{50} values (Fig. 2). Thus, the reduction with L-NNA was 0.63–0.83 $\log_{10}$ units in nondiabetic and LA-treated diabetic groups ($p < .001$), corresponding to 4.3–6.8-fold attenuation of sensitivity to ACh. In contrast, the (-log)EC_{50} for the diabetic group in the absence of L-NNA was reduced compared to the other groups ($p < .001$), being similar to their values in the presence of L-NNA; any further attenuation by L-NNA in diabetic rats was not statistically significant. The values in Fig. 2 were estimated from sigmoid curves for pooled data from all rats in each group because under the L-NNA condition the level of vasorelaxation was too low in 4/12 of the diabetic rats to permit individual estimates of (-log)EC_{50}. However, conventional estimates of group (-log)EC50 for nondiabetic, LA-treated nondiabetic, LA-treated diabetic (all rats), and diabetic ($n = 8$) groups were in close agreement and statistically indistinguishable from the values obtained using the pooled data

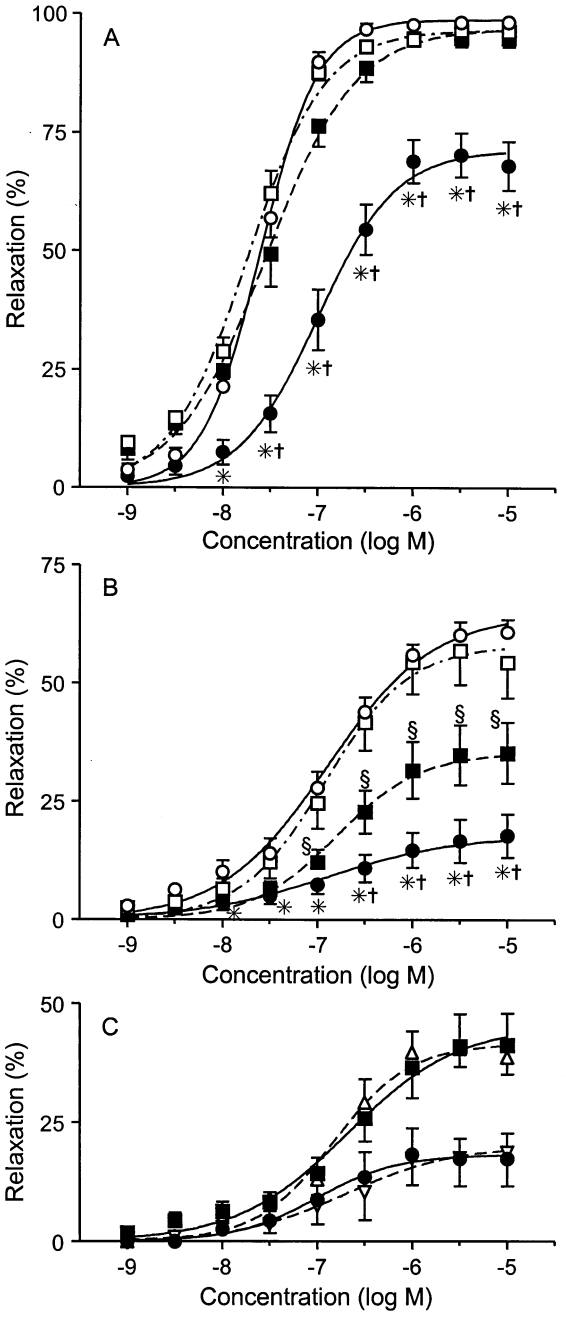

Fig. 1. Effects of diabetes and chronic α-lipoic acid treatment on acetylcholine concentration-response curves for endothelium-dependent vasodilation of the phenylephrine-preconstricted (3–100 μM) mesenteric vascular bed in the absence, (A), and presence, (B), of nitric oxide synthase inhibition (1–10 mM N^G -nitro-L-arginine). (C) Effects of joint cyclooxygenase and nitric oxide synthase inhibition in subgroups of untreated and treated diabetic rats. (A) and (B); nondiabetic control (○, solid line, $n = 12$), 8 week diabetic control (●, solid line, $n = 12$), 300 mg/kg LA-treated 8 week diabetic (■, dashed line, $n = 12$), LA-treated nondiabetic (□, dot-dashed line, $n = 12$) groups. (C); 8 week diabetic control ($n = 6$) before (●) and after (▽, dashed line) or LA-treated 8 week diabetic group ($n = 6$) before and after (△, dashed line) 30 min cyclooxygenase inhibitor pretreatment with flurbiprofen (5 μM). Data are group means ± SEM. *$p < .05$, diabetic control vs. nondiabetic control group; †$p < .05$, diabetic control vs. LA-treated diabetic group; §$p < .05$, LA-treated diabetic vs. nondiabetic control group.

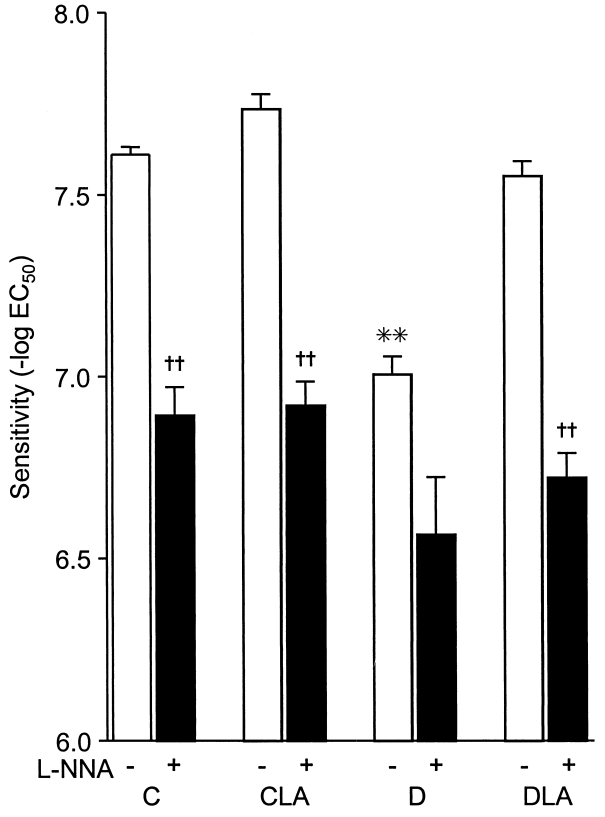

Fig. 2. Effects of diabetes and chronic α-lipoic acid treatment on acetylcholine (-log)EC_{50} values for endothelium-dependent vasodilation of the phenylephrine-preconstricted (3–100 μM) mesenteric vascular bed in the absence (open bars) and presence (filled bars) of the nitric oxide synthase inhibitor, N^G-nitro-L-arginine (L-NNA; 1–10 mM). C = nondiabetic control; CLA = LA-treated nondiabetic; D = 8 week diabetic control; DLA = LA-treated 8 week diabetic. Data are group means + SEM; group *n* values as for Fig. 1 A, B. $^{**}p < .001$, D vs. all other groups in the absence of L-NNA; $\dagger\dagger p < .001$, pre vs. post L-NNA treatment.

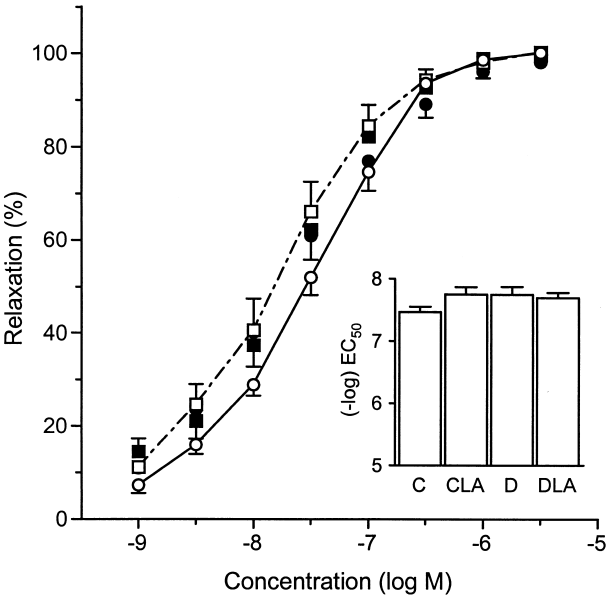

Fig. 3. Effects of diabetes and chronic α-lipoic acid treatment on sodium nitroprusside concentration-response data for endothelium-independent vasodilation of the phenylephrine-preconstricted (3–10 μM) mesenteric vascular bed in the presence of nitric oxide synthase inhibition (1–10 mM N^G-nitro-L-arginine). The inset histogram shows (-log)EC_{50} values. Nondiabetic control (○, C; $n = 12$), LA-treated nondiabetic (□, CLA; $n = 10$) 8 week diabetic control (●, D; $n = 12$), LA-treated 8 week diabetic (■, DLA; $n = 12$) groups. Data are group means ± SEM.

approach. In contrast to the data for ACh, maximum relaxation and sensitivity of the phenylephrine-preconstricted mesenteric bed to sodium nitroprusside (Fig. 3) in the presence of L-NNA, were not significantly altered by diabetes or LA treatment.

Pressor responses to phenylephrine (Fig. 4A) revealed that diabetes caused a 34.4 ± 11.4% ($p < .05$) lower maximum pressure development and a 0.42 ± 0.12 $\log_{10}$ unit reduction ($p < .05$) in sensitivity (Fig. 5), evidenced by (-log)EC_{50} values. In nondiabetic rats, LA treatment did not affect these parameters, however, with diabetes there was a marked attenuation of maximum pressure development compared to diabetic control ($p < .05$) and nondiabetic ($p < .001$) groups. In the presence of L-NNA, pressor responses (Fig. 4B) were elevated ($p < .001$), the largest increase being for LA-treated diabetic rats, such that group maximum values were not significantly different. Values for (-log)EC_{50} (Fig. 5) were also increased by L-NNA ($p < .01$) and did not differ significantly between groups.

Study 2: superior cervical ganglion blood flow and nociception responses

Eight weeks of diabetes caused a 52.2 ± 3.6% reduction ($p < .001$) in SCG blood flow (Fig. 6A). Intervention LA treatment over the final 2 weeks dose-dependently increased blood flow, with a log ED_{50} of 1.647 ± 0.008, corresponding to a daily dose of 44 mg/kg. The lowest dose employed (20 mg/kg) did not significantly alter blood flow. The maximum dose (100 mg/kg) corrected the perfusion deficit by 92.9 ± 5.5% ($p < .001$); values were not significantly different from that of the nondiabetic group. Mean systemic blood pressures (Fig. 6B) during hydrogen clearance recordings did not differ significantly between groups.

Before SCG blood flow recordings, nociceptive responses were measured in the 100 mg/kg LA-treated diabetic group. Mechanical thresholds (Fig. 7A) to evoke the foot withdrawal reflex were 15.2 ± 3.5% reduced ($p < .05$) by 6 weeks of diabetes, suggesting mechanical hyperalgesia. LA-treatment of these rats for 2 weeks increased thresholds ($p < .05$) such that they were within the nondiabetic range. Latencies for foot withdrawal to

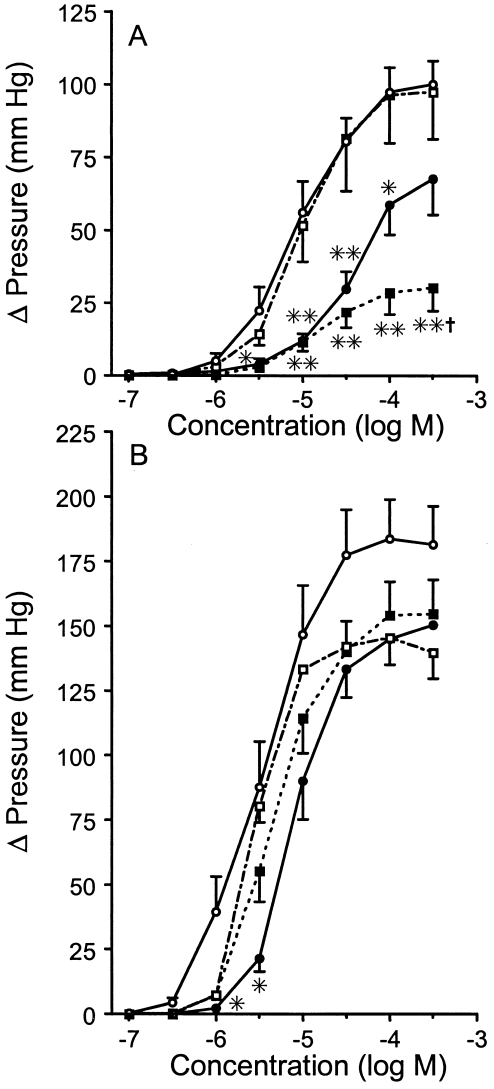

Fig. 4. Effects of diabetes and chronic α-lipoic acid treatment on phenylephrine pressor concentration-response curves for the mesenteric vascular bed in the absence (A) and presence (B) of nitric oxide synthase inhibition. Nondiabetic control (○, solid line, $n = 12$), 8 week diabetic control (●, solid line, $n = 12$), 300 mg kg^{-1} day^{-1} LA-treated 8 week diabetic (■, fine dashed line, $n = 6$), LA-treated nondiabetic (□, dot-dashed line, $n = 6$) groups. Data are group means ± SEM. $^{**}p < .001$, $^{*}p < .05$ vs. nondiabetic control group; †$p < .05$ LA-treated diabetic vs. diabetic control group.

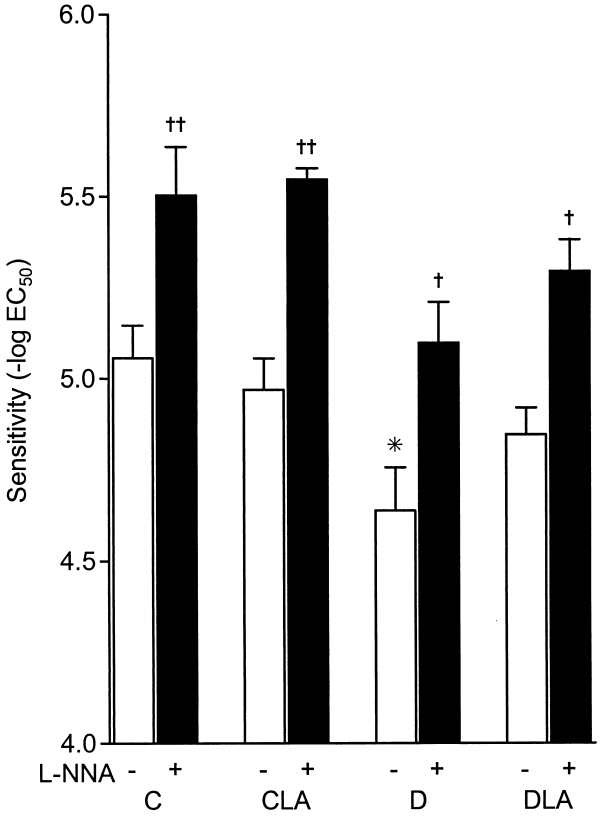

Fig. 5. Effects of diabetes and chronic α-lipoic acid treatment on phenylephrine pressor (-log)EC_{50} values for the mesenteric vascular bed in the absence (open bars) and presence (filled bars) of the nitric oxide synthase inhibitor, N^G-nitro-L-arginine (L-NNA). C = nondiabetic control; CLA = LA-treated nondiabetic; D = 8 week diabetic control; DLA = LA-treated 8 week diabetic. Data are group means + SEM; group n values as for Fig. 4. $^{*}p < .05$, D vs. C; ††$p < .001$, †$p < .01$, pre- vs. post-L-NNA treatment.

noxious thermal stimulation (Fig. 7B) were 26.8 ± 5.5% ($p < .001$) reduced by untreated diabetes, indicating thermal hyperalgesia. However, with LA treatment, mean latency increased ($p < .001$) to a value that was 18.1 ± 4.1% supernormal ($p < .01$).

DISCUSSION

The data show that diabetes and LA treatment had marked vascular effects. The diabetic deficit in endothelium-dependent relaxation of mesenteric vessels is in agreement with several studies using this tissue [13,29, 34,35], as well as on other vessels and vascular beds [7,12,20,22,36], including peripheral nerve [9,10]. Diabetic defects in vasodilation affected both NO and EDHF systems; evident for the former in terms of sensitivity to ACh-induced responses, and for the latter when NO and prostanoid systems were blocked. LA treatment largely prevented the development of the NO deficit and partially attenuated that for EDHF. The protective effect of antioxidant treatment on the NO system is in agreement with several previous studies using scavengers and transition metal chelators [12,20–22,27,36], however one study on isolated mesenteric vessels did not show beneficial functional effects of vitamin E [23]. The reason for this discrepancy is unclear, although the doses of that scavenger were somewhat lower than those necessary to have marked vascular effects in vasa nervorum and aorta of diabetic rats [20,37,38]. In previous studies, LA was

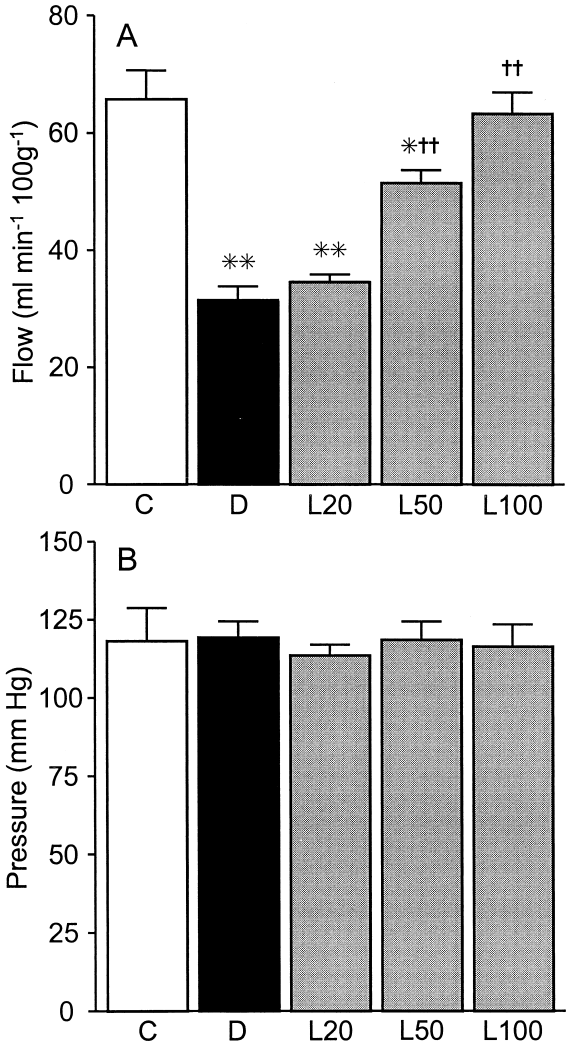

Fig. 6. Effects of diabetes and α-lipoic acid treatment on superior cervical ganglion nutritive blood flow (A) and mean systemic blood pressure (B). C = nondiabetic control ($n = 10$); D = 8 week diabetic control ($n = 8$); L20, L50, L100 = 8 week diabetic rats treated with 20 mg/kg ($n = 10$), 50 mg/kg ($n = 9$), or 100 mg/kg ($n = 10$) LA for the final 2 weeks. Data are group means + SEM. $^{**}p < .001$, $^{*}p < .05$ vs. C; $\dagger\dagger p < .001$ vs. D.

approximately 9-fold more efficacious than vitamin E in correcting diabetic neurovascular defects [26].

The diabetic effect on the mesenteric NO-mediated component of ACh relaxation was not caused by a reduced vascular smooth muscle NO sensitivity because sodium nitroprusside responses were not altered. This is in agreement with findings using nitrodilators in most experimental studies [3,4,10,20–22,27,29,34–36]. Thus, the diabetic defect appears to be at the level of impaired endothelial NO production or, as the LA effect could suggest, increased NO neutralization by ROS. The possibility of ACh receptor/signaling abnormalities cannot be completely excluded, although in other preparations diabetic NO deficits were seen for noncholinergic agonists or when a Ca^{2+} ionophore was used to bypass receptor mechanisms [6,39].

There have not been any previous reports on the effects of antioxidant treatment on the diabetic EDHF deficit. However, in a recent study using the mesenteric vascular preparation, an aldose reductase inhibitor had a qualitatively similar, albeit less marked, effect on EDHF [29]. This is compatible with the present results as aldose reductase inhibition has an indirect antioxidant action in diabetes, restoring a deficit in glutathione synthesis [40]. The chemical identity of EDHF is not known; indeed it is likely that there may be several mediators. To date, candidates include one or more cytochrome P450-derived arachidonic acid metabolites, an endocannabinoid, or K^+. EDHF action involves the opening of K^+ channels, and gap junctions may be important in the propagation of its effects [18,41–44]. The present data do not shed further light on the identity of EDHF. The precise role of ROS in the diabetic deficit is difficult to deduce from currently available evidence; actions at the level of EDHF synthesis/release, gap junction integrity, transduction, and K^+ channel modulation are possible and require further investigation. The balance between NO and EDHF-mediated vasodilation alters as vessel size decreases, EDHF predominating in the smaller resistance vessels controlling nutritive perfusion [16]. Therefore, the data stress the importance of ROS in the etiology of diabetic microvascular disease, in addition to macrovascular/atherosclerotic effects.

Diabetes caused a modest reduction in phenylephrine pressor action. This is probably explicable by mesenteric vascular hypertrophy, in part a response to the blood flow demands of diabetes-induced hyperphagia [45,46] causing an increase in vascular conductance. There was also a small decrease in sensitivity to phenylephrine, which could be due to a related adaptation. Reduced responses to agonists such as norepinephrine and angiotensin II have previously been observed in the mesenteric vascular bed of diabetic rats [45]; the data for LA treatment suggest that this phenomenon does not depend crucially on ROS involvement. However, there was a marked inhibition of phenylephrine pressor responses specifically in the LA-treated diabetic group, which was abolished by L-NNA. It is plausible that an important antioxidant action of LA was to scavenge endothelial ROS, preventing the neutralization of NO so that flow-induced NO liberation was unopposed. The constant flow conditions used in these experiments revealed that this was sufficient to overcompensate for a diabetic defect in the flow-induced NO mechanism [47].

The diabetes and LA treatment-induced changes seen for the mesenteric vasculature are likely to be present in the blood vessels supplying peripheral nervous tissue [9,10]. Previous studies have shown that several antioxi-

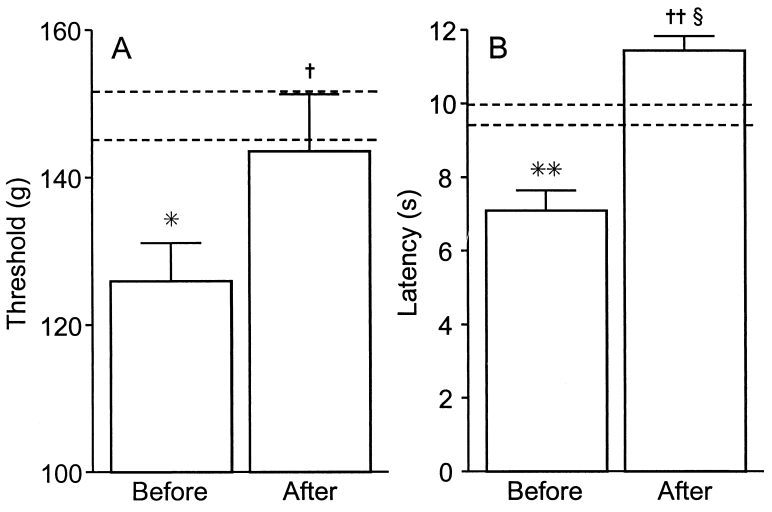

Fig. 7. Effects of diabetes and α-lipoic acid treatment on mechanical thresholds for the foot withdrawal reflex (A) and on the latency for foot withdrawal from noxious thermal stimulation (B). The dashed lines are the envelope of mean ± SEM for the nondiabetic control group ($n = 10$) and the columns are means + SEM for the diabetic group before and after 2 weeks of 100 mg/kg LA treatment ($n = 10$). $^{**}p < .001$, $^{*}p < .05$, effects of diabetes compared to nondiabetic control group; $\dagger\dagger p < .001$, $\dagger p < .05$, effects of LA treatment; $\S p < .01$, LA-treated diabetic vs. nondiabetic control group.

dants, including LA, prevent or correct sciatic nerve blood flow deficits in diabetic rats [25,26,37,38], and that chronic NO synthase inhibition attenuates this beneficial action [48]. The profound reduction in SCG blood flow in diabetic rats was dose-dependently corrected by LA treatment. The ED_{50}, at approximately 40 mg/kg, is in good agreement with previous reports of ED_{50} for prevention of sciatic nerve endoneurial ischemia and correction of sciatic conduction velocity deficits [25,26]. There is only a single previous report of reduced SCG perfusion, noted after 12 months of diabetes [28]. The present data are in agreement with the magnitude of this effect, and show that the diabetic deficit in ganglion blood flow occurs considerably earlier than 12 months. Thus, it is plausible that the impaired blood supply to cell bodies could contribute to the early incidence of autonomic neuropathy in experimental diabetes, which affects the innervation of diverse organs including the heart, gastrointestinal tract, blood vessels and corpus cavernosum [27,36,49–52]. Reduced perfusion is known to play a role at the very least for cardiac autonomic dysfunction, where vasodilator treatment improved function in both diabetic rats and man [50,53]. Impaired blood flow to neuronal cell bodies would restrict the metabolic energy supply necessary to synthesize and transport components essential for the maintenance of axonal integrity and neurotransmission. SCG phosphocreatine levels were reduced in diabetic rats and this was completely normalized by LA treatment [54]. LA and other antioxidants improved the function of corpus cavernosum autonomic innervation [27,36].

A parallel argument can be advanced for a potential contribution of a ROS/impaired ganglionic perfusion mechanism to sensory neuropathy, as dorsal root ganglia have similar blood flow deficits to SCG in diabetic rats [28]. Previous investigations have shown that LA treatment protects/improves sensory conduction velocity, mediated by large diameter myelinated nerve fibers [25,26]. Moreover, LA has been shown to be highly effective in protecting neural tissues against hyperglycemia-induced oxidative stress both in vivo and in vitro [25,55]. The present study extends the knowledge base of ROS/LA effects in diabetes to small somatosensory fiber systems and nociception responses, although the behavioral data do not discriminate between peripheral and central nervous system components.

The mechanical hyperalgesia of diabetes has been noted in several studies on different experimental hyperglycemic models [56,57]. These include galactose-fed rats where ROS production is increased, antioxidant defenses are reduced by polyol pathway activity, and endothelium-dependent vasorelaxation is impaired, in the absence of changes in insulin level or glucose homeostasis [58,59]. Nerve conduction deficits in galactosemic rats are corrected by antioxidants [60]. Thus, taken together with the LA treatment data, there is a strong case for ROS involvement in mechanical hyperalgesia. A recent clinical trial of LA showed improvements in symptomatic neuropathy, including pain perception [61].

These diabetic neural changes may be mediated in part via protein kinase C, entailing vascular and perhaps nonvascular mechanisms. Oxidative stress activates protein kinase C in diabetes and ischemia/reperfusion models [62,63]. Vascular protein kinase C is stimulated in

both experimental diabetes and galactosemia [64]. Protein kinase C inhibitors improve nerve conduction and blood flow in diabetic rats [65,66]. Electrophysiological studies of mechanosensitive nociceptor primary afferent fibers to skin showed altered responses to stimulation in diabetic rats, which were acutely corrected by local cutaneous injection of a protein kinase C inhibitor [67]. Knockout mice experiments have emphasized the importance of protein kinase Cγ in neuropathic pain [68].

Changes in responses to thermal stimulation are more varied in diabetic rat models. In streptozotocin-induced diabetes in young rats, there was hyposensitivity using the tail flick test [69]. However, in other studies in older rats, as in the present investigation, and in a model of type II diabetes, thermal hyperalgesia was observed [70, 71]. This was more than corrected by LA treatment. The mechanism is unclear; there have not been any reports of ROS involvement in the actual transduction process for thermal nociception. Blood flow changes could potentially contribute to LA's action both by direct effects on nerve and ganglion and perhaps by altering cutaneous circulation. Given the marked action on mesenteric vasculature it is possible that skin blood flow is elevated by LA treatment of diabetic rats, which could dissipate heat from the thermal stimulus, therefore delaying reflex foot withdrawal. This might explain the relative hyposensitivity induced by LA in diabetic rats, however, such a hypothesis is speculative at present and requires further detailed evaluation. Impaired blood flow appears to be involved in hypersensitivity to potentially noxious stimuli because allodynia is attenuated by vasodilator treatment in diabetic rats [72]. Furthermore, the traditional Japanese medicine Gosha-jinki-gan, which has some antioxidant actions, decreased hyperalgesia in the paw pressure test in diabetic mice; an effect that was reduced by local topical administration of a NO synthase inhibitor [73]. Thus, for nociception, the data are compatible with a vascular action of LA but do not rule out additional oxidative stress-related direct neural effects.

In conclusion, treatment of diabetic rats with the antioxidant LA had marked beneficial effects on both NO- and EDHF-mediated endothelium-dependent relaxation of mesenteric vasculature. This may be at least partly attributable to protection against the deleterious effect of ROS on vascular endothelium integrity. The effects of diabetes and LA treatment on SCG blood flow may depend on similar mechanisms and these may also be important for small nerve fiber functions such as nociception. Thus, the use of antioxidants like LA might constitute a potential therapeutic approach to diabetic neuropathy and vasculopathy.

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