Effects of DL-α-Lipoic Acid on Peripheral Nerve Conduction, Blood Flow, Energy Metabolism, and Oxidative Stress in Experimental Diabetic Neuropathy

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Experimental diabetic peripheral neuropathy (DPN) is marked by impaired nerve conduction velocity (NCV), reduced nerve blood flow (NBF), and a variety of metabolic abnormalities in peripheral nerve that have been variously ascribed to hyperglycemia, abnormal fatty acid metabolism, ischemic hypoxia, and/or oxidative stress. Some investigators propose that NCV slowing in experimental DPN can be explained entirely on the basis of nerve energy depletion secondary to reduced NBF. This article reports highly selective effects of administration of the antioxidant DL-α-lipoic acid (LA) to streptozotocin-injected diabetic rats. LA improved digital sensory but not sciatic-tibial motor NCV, corrected endoneurial nutritive but not composite NBF, increased the mitochondrial oxidative state without correcting nerve energy depletion, and enhanced the accumulation of polyol pathway intermediates without worsening *myo*-inositol or taurine depletion. These studies implicate oxidative stress as an important pathophysiological factor in experimental DPN. They reveal complex interrelationships among nerve perfusion, energy metabolism, osmolyte content, conduction velocity, and oxidative stress that may reflect the heterogeneous and compartmentalized composition of peripheral nerve. *Diabetes* 49:1006–1015, 2000

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AGE, advanced glycation end product; α-KG, α-ketoglutarate; AR, aldose reductase; β-HB, β-hydroxybutyrate; CDNB, 1-chloro-2,4-dinitrobenzene; Cr, creatinine; DHAP, dihydroxyacetone phosphate; DPN, diabetic peripheral neuropathy; GSH, reduced glutathione; GSHTrans, glutathione transferase; GSSG, glutathione disulfide; GSSGRed, glutathione reductase; LA, DL-α-lipoic acid; MNCV, motor nerve conduction velocity; NBF, nerve blood flow; NCV, nerve conduction velocity; ND, nondiabetic; ND/L, LA-treated nondiabetic; NGF, nerve growth factor; PCr, phosphocreatinine; ROS, reactive oxygen species; SNCV, sensory nerve conduction velocity; SOD, superoxide dismutase; STZ, streptozotocin; STZ-D, STZ-injected diabetic; STZ-D/L, LA-treated STZ-injected diabetic.

Diabetic peripheral neuropathy (DPN) is a multifactorial disorder arising from hyperglycemia and/or insulin deficiency; it is characterized by a complex pathogenetic network of interrelated metabolic, neurotrophic, and vascular defects (1). These defects initiate chronic insidious progressive damage and loss in unmyelinated and myelinated peripheral nerve fibers, which culminate in a distal symmetric primarily sensory peripheral polyneuropathy. The nature and precise cellular localization of the specific metabolic consequences of hyperglycemia that initiate the pathogenetic cascade remain highly speculative, and they are somewhat confounded by the complex, composite, and multicellular nature of peripheral nerve (1). Recently, oxidative stress has been invoked as a critical factor in the development of DPN (1–8). Hyperglycemia is thought to promote oxidative stress through both nonenzymatic and enzymatic mechanisms. Nonenzymatic protein glycation is thought to generate reactive oxygen species (ROS) through a complex series of chemical and cellular intermediates (1). Reduction of glucose to sorbitol by the nicotinamide dinucleotide–linked enzyme aldose reductase (AR) is also thought to promote oxidative stress (1,8) by shifting the $NADP^+$/NADPH and glutathione redox couples toward the more oxidized forms. Moreover, accumulation of sorbitol produces reciprocal depletion of taurine, an intracellular osmolyte and an endogenous antioxidant (9), compromising antioxidative defense (1,7,9). Accumulation of conjugated dienes (10) and reductions in superoxide dismutase (SOD) (10), glutathione peroxidase (11), and taurine (12) are regarded as evidence for increased oxidative damage and reduced oxidative defense in experimental DPN (1).

Antioxidant therapy with aqueous- or lipid-soluble free radical scavengers or metal chelators improves nerve conduction velocity (NCV) in experimental DPN (5–7,13). Antioxidant therapy also ameliorates deficits in both neuropeptide expression (2) and nerve blood flow (NBF) (5), with the latter implicated as causing endoneurial hypoxia (14) and reduced NCV in diabetic rats. Impaired NBF in experimental DPN has been ascribed to defects in prostanoid and/or nitric oxide metabolism (15–17), both of which are potentially attributable in part to the presence of ROS (1,5–8). Advanced glycation end products (AGEs) alter

endothelial function in diabetes (18) and induce oxidative stress in cultured endothelial cells (19), where they deplete reduced glutathione (GSH) and activate nuclear factor-κB (19,20). ROS also have direct neurotoxic activity, promoting neuronal apoptosis (21,22) and mediating ischemia-reperfusion injury in the nervous system (23). Conversely, neurotrophic factors protect neurons against oxidative stress (24) and upregulate antioxidative defense mechanisms (24,25). Thus, the interrelationships among hyperglycemia, the AR pathway, oxidative stress, endoneurial hypoxia, nerve energy depletion, impaired neurotrophic support, and slowed NCV in experimental DPN may be quite intricate (1). However, a more simplified paradigm views pathophysiologically relevant oxidative stress as being confined to the endoneurial vasculature; attributes downstream effects on NCV almost entirely to endoneurial ischemic hypoxia, impaired oxidative metabolism, and nerve energy depletion (26); and, thus, predicts that these parameters should respond synchronously to antioxidant treatment.

α-Lipoic acid is an endogenous free radical scavenger (8,27) that also activates glucose uptake and the pyruvate dehydrogenase complex and serves as a metabolic substrate for the E3 pyruvate dehydrogenase component (1,27). DL-α-Lipoic acid (LA) administration has also been reported to ameliorate some of the symptoms of DPN in diabetic patients (28) and to improve endoneurial nutritive NBF and NCV in diabetic rats (8,29). Treatment with LA or other antioxidants protects cultured endothelial cells against the oxidative stress induced by AGEs (18,20). This study used LA treatment to explore the relationships between NBF and NCV deficits and between altered nerve metabolism and bioenergetics in experimental DPN. The results suggest that some of these interrelationships are considerably more complex than previously recognized, with LA producing equally selective and divergent effects on nerve polyol and energy metabolism, NBF, sensory NCV (SNCV), and motor NCV (MNCV).

RESEARCH DESIGN AND METHODS

Animal model. Barrier-sustained Cesarean-delivered male Wistar rats (200–300 g) were fasted overnight and then injected with 50 mg/kg i.p. streptozotocin (STZ) (Upjohn, Kalamazoo, MI) in 0.2 ml of 10 mmol/l citrate buffer (pH 5.5). Diabetes was defined as a nonfasting plasma glucose level >200 mg/dl in tail vein blood (One Touch II; LifeScan, Milpitas, CA) 48 h after STZ injection and again on the day before death. Uninjected nondiabetic (ND)and STZ-injected diabetic (STZ-D) rats were maintained in individual air-filtered metabolic cages with ad libitum access to water, and they were fed a standard synthetic laboratory diet (ICN; Biomedicals, Cleveland, OH) (12). ND and STZ-D rats were randomly assigned to receive no treatment or, after 48 h, to begin receiving 100 mg · kg^{-1} · day^{-1} i.p. LA (Sigma, St. Louis, MO) as an aqueous solution. This dose of LA has been shown previously to completely correct endoneurial nutritive NBF in STZ-D rats (8). All end point measurements were performed 6 weeks later, after reconfirmation of diabetes, by investigators unaware of the treatment group assignment (however, 6-week STZ-D rats were often distinguishable from ND rats by inspection).

A total of 3 experiments were conducted. In experiment 1, the rats were anesthetized with intraperitoneal urethane (1–1.2 g/kg); SNCV, MNCV, and composite NBF (laser Doppler) were measured. The rats were then killed, and the left and right sciatic nerves were rapidly excised and cleaned to measure the sorbitol pathway intermediates, *myo*-inositol, taurine, GSH, glutathione disulfide (GSSG), and ascorbate and to measure the activities of SOD, catalase, glutathione transferase (GSHTrans), glutathione reductase (GSSGRed), and (Na,K)-ATPase enzymes. In experiment 2, the LA-treated ND (ND/L) group was omitted, based on the results from the first study, and endoneurial nutritive NBF was measured by the H_2 clearance method (14). In experiment 3, which was specifically designed for measurement of nerve NAD-redox and energy status, femoral segments of sciatic nerves were quickly removed from animals that were initially sedated with inhaled CO_2 (10–15 s) and then rapidly killed by cervical dislocation. Exposure to CO_2 was performed in a specially designed 2-compartment plastic chamber, the lower of which contained dry ice and was separated from the upper compartment (used as a rat container) by a septum perforated with numerous small holes. Such rapid sampling (the total elapsed time between initiation of CO_2 exposure and removal and freezing of nerve samples was 30 s) was used because of the rapid decay of high-energy phosphate compounds in peripheral nerve that begins once the circulation is interrupted (30). The rapid sampling and analytical techniques used in the current studies readily detected the anticipated improvement in the ATP:ADP and phosphocreatinine (PCr)-to-creatinine (Cr) ratios of STZ-D rat sciatic nerve in response to vasodilator treatment (I.O., M.J.S., D.A.G., unpublished data). The second nerve was used for measurement of total quinone reductase and NADH diaphorase activities.

Validation studies to assess this rapid-sampling methodology, in comparison with more generally used anesthetic protocols (3–5,8), considered the theoretical possibility that the 10–15 s CO_2 exposure necessary for CO_2 narcosis would produce a respiratory acidosis sufficient to decrease systemic and intracellular pH to the point at which reduced metabolites of NAD(P) dehydrogenase reactions (NAD[P] collectively refers to NAD, NADH, NADP, and NADPH) would accumulate and decrease the free NAD^+:NADH ratios in the cytosol and mitochondria of peripheral nerve. This decreased free NAD^+:NADH ratio would be expected to produce a secondary depletion in high-energy phosphates because of the following relationship:

$$\text{Cytosolic } \frac{[\text{ATP}]}{[\text{ADP}] \times [\text{Pi}]} =$$

$$\text{cytosolic } \frac{[\text{NAD}^+]}{[\text{NADH}]} \times \frac{[\text{glyceraldehyde 3-phosphate}] \times K}{[\text{3-phosphoglycerate}]}$$

where K is the combined equilibrium constant of the glyceraldehyde 3-phosphate dehydrogenase and the triose phosphate isomerase systems. Alternatively, slow induction with general anesthetics might diminish higher-energy phosphates in tissues, such as nerve (which lacks circulatory autoregulation), by compromising cardiorespiratory regulation. Sciatic nerves from 11 ND rats that were killed by cervical dislocation after CO_2 narcosis exhibited increased levels of high-energy phosphates and NAD^+:NADH ratios and lower lactate levels than nerves from conventionally anesthetized ND rats (3–5,8) (Table 1). The fact that lactate and malate did not accumulate in the CO_2-sedated rats versus the decapitated rats (Table 1) implied that H^+ accumulation, with an accompanying shift in the equilibrium of the cytosolic NAD(P)-dependent dehydrogenase reactions, does not occur in peripheral nerve within the 10–15 s exposure to CO_2. These data demonstrate that the 30-s interval between initiation of CO_2 narcosis and tissue removal outweighs any theoretical disadvantage from respiratory acidosis in the preservation of labile nerve high-energy metabolites, thereby suggesting that anesthesia should be avoided in such experiments.

Biochemical measurements

Antioxidative defense enzymes. For measurements of antioxidative defense enzyme activities, ~30 mg of frozen sciatic nerves were homogenized in 1 ml ice-cold 0.1 mol/l sodium phosphate buffer, pH 6.5. Homogenates were centrifuged at 20,000*g*, and the supernatant fraction was used for assays of enzymatic activities and protein content. Protein levels were quantified with Pierce BCA protein assay kits (Rockford, IL). SOD activity was measured by spectrophotometrically monitoring (at 480 nm) the auto-oxidation of (–)-epinephrine at pH 10.4 for ~5 min. The reaction mixture contained 0.8 ml of 50 mmol/l glycine buffer, pH 10.4, and 0.2 ml supernatant. The reaction was initiated by the addition of 0.02 ml of a 20 mg/ml solution of (–)-epinephrine. Due to its poor solubility, (–)-epinephrine (40 mg) was suspended in 2 ml water and was solubilized by adding 2–3 drops of 2N HCl. SOD activity was expressed as nmol of (–)-epinephrine protected from oxidation by the sample compared with the corresponding readings in the blank cuvette. The molar extinction coefficient of 4.02 mmol · l^{-1} · cm^{-1} was used for calculations. Catalase activity was measured spectrophotometrically by monitoring for 5 min the decrease in absorbance at 240 nm after addition of 0.1 ml of supernatant to 0.9 ml of a solution of H_2O_2 in 50 mmol/l phosphate buffer, pH 6.8. The starting concentration of H_2O_2 was sufficient to produce an absorption of ~1.0 optical density units. The enzyme activity was calculated using 2.04 mmol · l^{-1} · cm^{-1} as the molar extinction coefficient. GSH transferase activity toward 1-chloro-2,4-dinitrobenzene (CDNB) was measured according to the methods described by Habig et al. (31). A reaction mixture of 0.8 ml contained 0.1 ml sodium phosphate buffer, pH 6.5, 1 mmol/l GSH, 1 mmol/l CDNB dissolved in ethanol, and 1 mmol/l EDTA. The reaction was initiated by the addition of 0.2 ml supernatant and was monitored spectrophotometrically at 340 nm for 5 min. Calculations were performed using an extinction coefficient of 9.6 mmol · l^{-1} · cm^{-1}. GSSG reductase activity was measured spectrophotometrically at 340 nm by moni-

TABLE 1
Effect of CO_2 narcosis and anesthesia on nerve metabolites

	CO_2 narcosis	Urethane*	Ketamine†	Phenobarbital†	Decapitation‡
n	11	6	6	6	10
ATP	0.887 ± 0.109	0.374 ± 0.205§	0.341 ± 0.207§	0.682 ± 0.269	0.804 ± 0.272
PCr	2.80 ± 0.12	0.877 ± 0.487§	0.691 ± 0.387§	1.10 ± 0.40§	2.45 ± 0.44
Lactate	1.50 ± 0.10	3.68 ± 2.03‖	3.68 ± 1.78‖	3.26 ± 0.71‖	1.86 ± 0.43
Pyruvate	0.173 ± 0.014	0.129 ± 0.047	0.122 ± 0.007	0.105 ± 0.013	0.142 ± 0.024
Malate	0.121 ± 0.022	—	—	—	0.131 ± 0.043
NAD^+/NADH	1,051 ± 66	407 ± 249§	356 ± 155§	299 ± 71§	871 ± 235

Data are *n* or means ± SD and are given in micromoles per gram wet weight. *The urethane dose was 1.0–1.2 g/kg i.p.; †the ketamine and phenobarbital doses were 100 mg/kg i.p.; ‡published data are shown for comparison only with the findings by Obrosova et al. (63); §$P < 0.01$ vs. CO_2; ‖$P < 0.05$ vs. CO_2.

toring NADPH oxidation coupled to reduction of GSSG to GSH for 5 min (32). The reaction was started by the addition of 0.2 ml sample to 0.8 ml reaction mixture containing 0.1 mol/l potassium phosphate buffer, pH 7.0, 2.5 mmol/l GSSG, and 125 µmol/l NADPH. Calculations were performed with a molar extinction coefficient of 6.22 mmol · l^{-1} · cm^{-1}. Total quinone reductase activity was measured spectrophotometrically at 340 nm. The reaction was initiated by the addition of 0.2 ml of sample to 0.8 ml of reaction mixture containing 50 mmol/l Tris-HCl, pH 7.5, 0.02% Tween-20, 0.3 mmol/l NADH, and 0.05 mmol/l p-benzoquinone. Calculations were performed with a molar extinction coefficient of 6.22 mmol · l^{-1} · cm^{-1}. Cytochrome b_5 reductase was measured using the same mixture containing dicoumarol (32). The difference between total quinone reductase and cytochrome b_5 reductase activities was regarded as DT-diaphorase.

Glycolytic and tricarboxylic acid cycle intermediates, glutamate, ammonia, ketone bodies, and parameters of energy metabolism. Femoral segments of sciatic nerve (~20 mg) were homogenized in 1 ml of 6% $HClO_4$. The levels of lactate, pyruvate, glutamate, α-ketoglutarate (α-KG), malate, ammonia, 3-phosphoglycerate, dihydroxyacetone phosphate (DHAP), β-hydroxybutyrate (β-HB), acetoacetate, ATP, ADP, PCr, and Cr were assayed in neutralized extracts spectrofluorometrically by the enzymatic methods described by Lowry and Passonneau (33).

Calculations of free NAD ratios. Direct measurements of NAD(P) (i.e., NAD, NADH, NADP, and NADPH) do not provide information on the compartmentalization of NAD(P) and do not separate free from protein-bound forms (only free fractions determine direction and free-energy changes of dehydrogenase reactions). In contrast, the indicator metabolite method, which was originally proposed by Williamson et al. (34) and was later used in many biochemical studies (35), assesses free $NAD(P)^+$:NAD(P)H ratios in the cytoplasmic and mitochondrial compartments. These ratios are calculated from the ratios of the concentrations of the oxidized and reduced forms of suitable NAD(P) cofactors for specific mitochondrial and cytoplasmic NAD(P)-linked dehydrogenase reactions. This approach permits separate assessment of ratios of free NAD(P) in the mitochondria and cytosol in the same deproteinized extract.

With this approach, free mitochondrial NAD^+:NADH ratios were calculated from metabolite concentrations and the equilibrium constant of β-HB dehydrogenase as follows (34):

$$\text{Mitochondrial } \frac{[NAD^+]}{[NADH]} = \frac{[\text{acetoacetate}]}{[\beta\text{-HB}]} \times \frac{1}{k_1}$$

where k_1 is the equilibrium constant of β-HB dehydrogenase (34). Serum acetoacetate and β-HB levels are markedly affected by STZ-D (36); therefore, free mitochondrial NAD^+:NADH ratios were also calculated from the equilibrium of the glutamate dehydrogenase system as follows (34):

$$\text{Mitochondrial } \frac{[NAD^+]}{[NADH]} = \frac{[\alpha\text{-KG}] \times [\text{ammonia}]}{[\text{glutamate}]} \times \frac{1}{k_2}$$

where k_2 is the equilibrium constant of glutamate dehydrogenase (34). Free cytosolic NAD^+:NADH ratios were calculated from the lactate dehydrogenase system as follows (34):

$$\text{Cytosolic } \frac{[NAD^+]}{[NADH]} = \frac{[\text{pyruvate}]}{[\text{lactate}]} \times \frac{1}{k_3}$$

where k_3 is the equilibrium constant of lactate dehydrogenase (34).

Nerve osmolytes and (Na,K)-ATPase activity. The content of sorbitol, fructose, and *myo*-inositol in deproteinized homogenates of sciatic nerve was determined by enzymatic spectrofluorometry with sorbitol dehydrogenase (37), fructose dehydrogenase (38), and *myo*-inositol dehydrogenase (39), respectively. Taurine was measured in sciatic nerve by reversed phase high-performance liquid chromatography after precolumn derivatization with o-phthalaldehyde (12) using glutamine as the internal standard. Samples were analyzed in a Waters system (Millipore, Milford, MA) equipped with a model 501 pump, a 717 autosampler, a 3.9 × 150 mm Nova-Pak C18 column, and a model 470 scanning fluorescence detector. For all osmolyte measurements, standard curves were generated daily, and the recovery-corrected values were expressed as nanomoles per milligram wet weight of tissue. Ouabain-sensitive (Na,K)-ATPase activity was measured in samples of rat sciatic nerve homogenized on ice in 2 ml 0.2 mol/l sucrose plus 0.02 mol/l Tris-HCl, pH 7.5, by 3 10-s bursts with a Polytron model PT 10-35 tissue grinder (Brinkman Instruments, Westbury, NY) (40). Aliquots of homogenate were assayed enzymatically for total ATPase activity in 1 ml reaction mixture containing 100 mmol/l NaCl, 10 mmol/l KCl, 2.5 mmol/l $MgCl_2$, 1 mmol/l Tris-ATP, 1 mmol/l phosphoenolpyruvate, 30 mmol/l imidazole-HCl buffer, pH 7.3, 0.15 mmol/l NADH, 50 µg lactate dehydrogenase, and 30 µg pyruvate kinase (40). After an initial stabilization period, activity was monitored spectrophotometrically at 340 nm for at least 15 min. Ouabain (20 µl of a 25 mmol/l solution) was added, and the activity was read for at least 15 min (40). Ouabain-inhibitable (Na,K)-ATPase activity was defined as the activity difference before and after adding ouabain and expressed as micromoles ADP formed per gram wet weight per hour (40).

Electrophysiological measurements. Sciatic-tibial MNCV, distal digital SNCV, and caudal NCV were measured after induction of anesthesia with intraperitoneal urethane (1–1.2 g/kg). Body temperature was monitored by a rectal probe and maintained at 37°C with a warming pad. Hind-limb skin temperature was also monitored by a thermistor and maintained between 36 and 38°C by radiant heat. The left sciatic-tibial motor conduction system was stimulated proximally at the sciatic notch and distally at the ankle via bipolar electrodes with supramaximal stimuli (8 V) at 20 Hz (36). The latencies of the compound muscle action potentials were recorded via bipolar electrodes from the first interosseous muscle of the hind-paw and were measured from the stimulus artifact to the onset of the negative M-wave deflection. MNCV was calculated by subtracting the distal latency from the proximal latency, and the result was divided into the distance between the stimulating and recording electrode. Hind-limb SNCV was recorded in the digital nerve to the second toe by stimulating with a square-wave pulse duration of 0.05 ms using the smallest intensity current; this procedure resulted in a maximal amplitude response. The sensory nerve action potential was recorded behind the medial malleolus. The distance between recording and stimulating electrodes was ~25 mm, and the distance between indifferent and active recording needles was maintained at 10 mm (41). Approximately 10 responses were averaged to obtain the position of the negative peak. The maximal SNCV was calculated from the latency to the onset of the initial negative deflection and the distance between stimulating and recording electrodes. Caudal NCV was measured in rat tail warmed to 37°C for 5 min in liquid paraffin using 2 pairs of steel stimulating needles positioned ~8 and 3 cm proximal to a distal pair, which were used to record compound muscle action potentials (42).

Measurements of sciatic NBF. In experiment 1, composite sciatic NBF was assessed in anesthetized rats using a laser Doppler blood flow monitor (MBF3/D; Moor Instruments, Devon, U.K.). The left carotid artery was cannulated and connected to a transducer to monitor mean systemic blood pressure, which was recorded and displayed continuously on a computer screen. The sciatic nerve was exposed via a small incision on the left flank, and the laser

probe (tip diameter 0.85 mm) was applied just in contact with an area of the sciatic trunk as free as possible from visible epi/perineurial vessels. Care was taken to avoid compressing the nerve. The exposed nerve was then covered with liquid paraffin to avoid tissue dehydration, and the flux was allowed to reach a stable baseline over a period of 10–15 min before readings were taken. Body temperature was maintained at 37°C as previously described, and the temperature in the vicinity of the nerve was maintained by placing the rat under a source of radiant heat. Biofeedback from the rat was derived from a thermistor placed near the exposed sciatic nerve and was set to maintain local temperature between 36 and 38°C. Four flux measurements were obtained from the same nerve segment, and the mean value over a duration of 2 min was calculated as arbitrary Doppler units. To take into consideration perfusion pressure variations, all flow data were expressed as vascular conductance calculated by dividing the blood flow by the mean systemic blood pressure over the recording period.

In experiment 2, nutritive NBF was assessed by H_2 clearance (14). Animals were anesthetized with an intraperitoneal injection of urethane (1 g/kg). The left carotid artery was cannulated with polyethylene tubing, and the patency was maintained with heparinized saline (50 U/ml normal saline). The catheter was connected to a transducer, and the blood pressure was monitored by a MacLab data acquisition system (ADInstruments, Castle Hill, New South Wales, Australia). A tracheotomy was performed, and the animal was artificially respired with O_2:N_2 (20:80%) using a small animal ventilator (Harvard Apparatus, South Natick, MA). Core body temperature was monitored by use of a rectal probe, and the hind-limb muscle temperature was measured by a needle probe inserted into the muscle layer; both were maintained at 37°C by radiant heat. The right sciatic nerve was exposed and gently dissected from the surrounding tissue. The skin around the incision was positioned to create a reservoir. A ground electrode was inserted subcutaneously into the flank of the rat. By use of a micromanipulator, an H_2-sensitive platinum electrode (tip diameter 0.2 μm) (World Precision Instruments, Sarasota, FL) was inserted into the nerve above the trifurcation. Mineral oil at 37°C was used to fill the reservoir and to prevent diffusion of gases from the nerve. The nerve was polarized with 0.25 V, and when a stable baseline was achieved, the animal received a gas mixture containing 10% H_2, which was continued until the current change stabilized (10–30 min), at which time H_2 flow was terminated. Current recordings were made every 30 s until baseline levels were achieved (30–60 min). After the experiment, mono- or biexponential clearance curves were fitted to the data (Graphpad Software, La Jolla, CA). Nutritive NBF was taken as the slow component of the curve. An average of 2 determinations at different sites was used to determine nutritive NBF.

Statistical analysis. Data are expressed as means ± SE. Differences among experimental groups were determined by analysis of variance, and the significance of differences between these groups was assessed by the Student-Newman-Keuls multiple range test. Significance was defined as $\alpha = 0.05$, except for multiple energy metabolite measurements that were used for the metabolite indicator method of assessing the energy state; in this case, $\alpha = 0.01$ to minimize the likelihood that these multiple individual determinations could attain significance by chance alone.

TABLE 2
Characteristics of experimental groups

	ND	ND/L	STZ-D	STZ-D/L
Experiment 1				
n	7	8	8	8
End wt (g)	439 ± 17	408 ± 18	347 ± 26*	332 ± 11*
Glucose (mg/dl)	74 ± 1	70 ± 6	286 ± 16†	297 ± 24†
Experiment 2				
n	12	0	12	9
End wt (g)	477 ± 9	ND	349 ± 19†	350 ± 10†
Glucose (mg/dl)	63 ± 2	ND	323 ± 9†	357 ± 14†
Experiment 3				
n	21	19	19	18
End wt (g)	455 ± 27	420 ± 27	327 ± 51	306 ± 32
Glucose (mg/dl)	65 ± 7	67 ± 9	340 ± 60	322 ± 44

Data are *n* or means ± SD. *P < 0.05 vs. ND and ND/L; †P < 0.01 vs. ND and ND/L within each experiment.

RESULTS

Effect of STZ-induced diabetes and LA on body weights and plasma glucose levels. Baseline body weights were similar in all treatment groups (data not shown). Plasma glucose values were >200 mg/dl in all STZ-D rats 48 h after STZ injection and at the end of the study. After 6 weeks, STZ-induced diabetes resulted in a significant decrease in body weight and a multifold increase in plasma glucose, as compared with ND controls in all 3 of the experiments (Table 2). Treatment for 6 weeks with LA was associated with small reductions in the body weight that did not achieve statistical significance (Table 2). LA did not affect plasma glucose values in any experimental group (Table 2).

Effects of STZ-induced diabetes and LA on NCV. Sciatic-tibial MNCV, caudal NCV, and digital SNCV were reduced in 6-week untreated STZ-D rats compared with those in untreated ND rats (Fig. 1*A* and *B*). Treatment with LA did not alter NCV measurements in ND rats (Fig. 1*A*) or MNCV or caudal NCV in STZ-D rats (Fig. 1*A* and *B*). However, LA treatment significantly improved digital SNCV (Fig. 1*A* and *B*), fully correcting this parameter in experiment 2 (Fig. 1*B*). Therefore, LA treatment selectively affected conduction slowing in distal sensory fibers, confirming an earlier report (8), but at

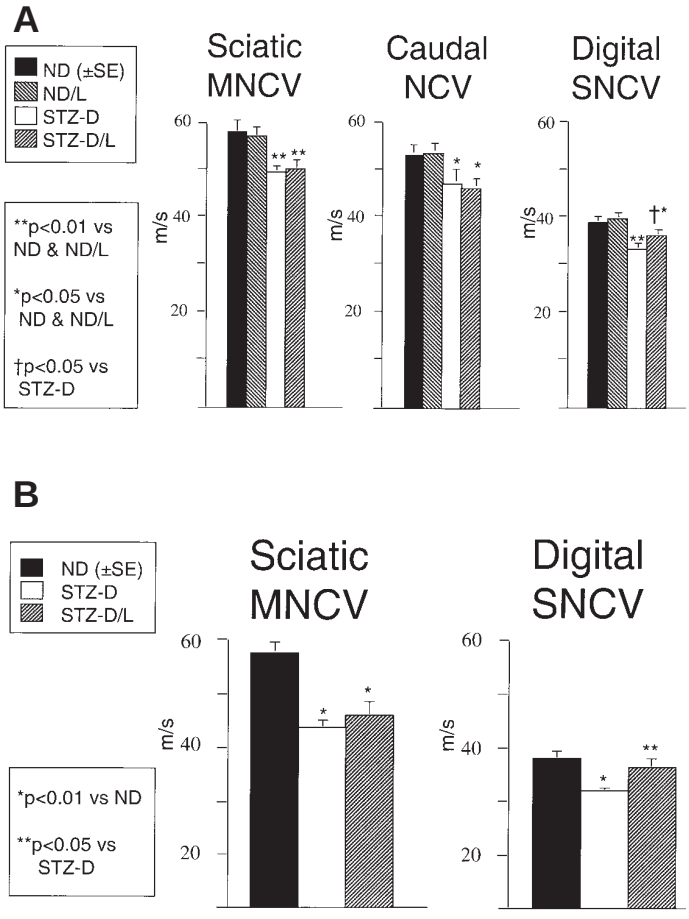

FIG. 1. Effect of STZ-induced diabetes and LA treatment on NCV. Sciatic MNCV, caudal NCV, and digital SNCV were determined in ND and STZ-D rats that were untreated or treated with LA for the full duration of 2 separate 6-week experiments, experiment 1 (*A*) and experiment 2 (*B*), as described in RESEARCH DESIGN AND METHODS. Caudal NCV was not determined in experiment 2.

variance to a more recent report (29), in which both saphenous SNCV and sciatic MNCV were improved with LA.

Effects of STZ-induced diabetes and LA on NBF. NBF, whether measured as composite flow by laser Doppler (43) or as nutritive endoneurial flow by H_2 clearance (14), was markedly reduced in STZ-D versus ND rats (Fig. 2*A* and *B*). These effects could not be attributed to changes in mean blood pressure (although mean blood pressure was significantly reduced in the STZ-D group in experiment 2 [Fig. 2*B*] but not in experiment 1 [Fig. 2*A*]): vascular conductance, which corrects for blood pressure changes, was reduced in STZ-D rats in both experiments (Fig. 2*A* and *B*). Composite NBF tended to be lower in ND rats treated with LA, but this effect was not statistically significant (Fig. 2*A*). Despite similar effects of STZ-induced diabetes on composite and nutritive NBF, LA had diametrically opposite effects on these 2 measures of NBF in STZ-D rats. LA tended to further depress composite NBF and vascular conductance in STZ-D rats, although this was not statistically significant (Fig. 2*A*). In contrast, LA improved endoneurial nutritive NBF and endoneurial vascular conductance in STZ-D rats, so that it was no longer different from that in ND rats (Fig. 2*B*).

Effects of STZ-induced diabetes and LA on nerve osmolyte levels. Nerve glucose, sorbitol, and fructose levels tended to be slightly increased in ND rats treated with LA versus untreated ND rats, but these differences did not achieve statistical significance (Fig. 3*A*). Nerve glucose, sorbitol, and fructose levels were markedly elevated in untreated STZ-D rats versus untreated ND rats (Fig. 3*A*). Despite the absence of an effect on blood glucose levels in STZ-D rats, LA further increased the already-elevated levels of glucose, sorbitol, and fructose in STZ-D rats by 1.9-, 1.6-, and 1.4-fold (all $P < 0.05$ vs. untreated STZ-D rats), respectively (Fig. 3*A*). Nerve *myo*-inositol content was unaffected by LA in ND rats, was decreased by 23% ($P < 0.05$) in untreated STZ-D rats, and, by treatment with LA, was increased by 13% to levels that were not statistically different from those of either untreated ND or STZ-D rats (Fig. 3*B*). Nerve taurine levels were similarly reduced in STZ-D rats and were corrected by LA (Fig. 3*B*). Therefore, despite increasing nerve glucose content and polyol pathway products in the STZ-D rats, LA paradoxically attenuated depletion of the other compatible osmolytes, *myo*-inositol and taurine (1,12,16,36).

Effects of STZ-induced diabetes and LA on nerve (Na,K)-ATPase activity. Total ATPase activity was reduced by 34% in sciatic nerve homogenates from untreated STZ-D rats ($P < 0.01$) (Fig. 3*B*). This decrease was specific for the ouabain-sensitive component (i.e., [Na,K]-ATPase activity) since ATPase activity measured in the presence of ouabain was not different in nerve homogenates from ND and STZ-D rats (data not shown). LA treatment increased (Na,K)-ATPase activity by 32% ($P < 0.05$) in STZ-D rats to levels not significantly different from those in ND rats. No effects of LA were observed on (Na,K)-ATPase activity in ND rats

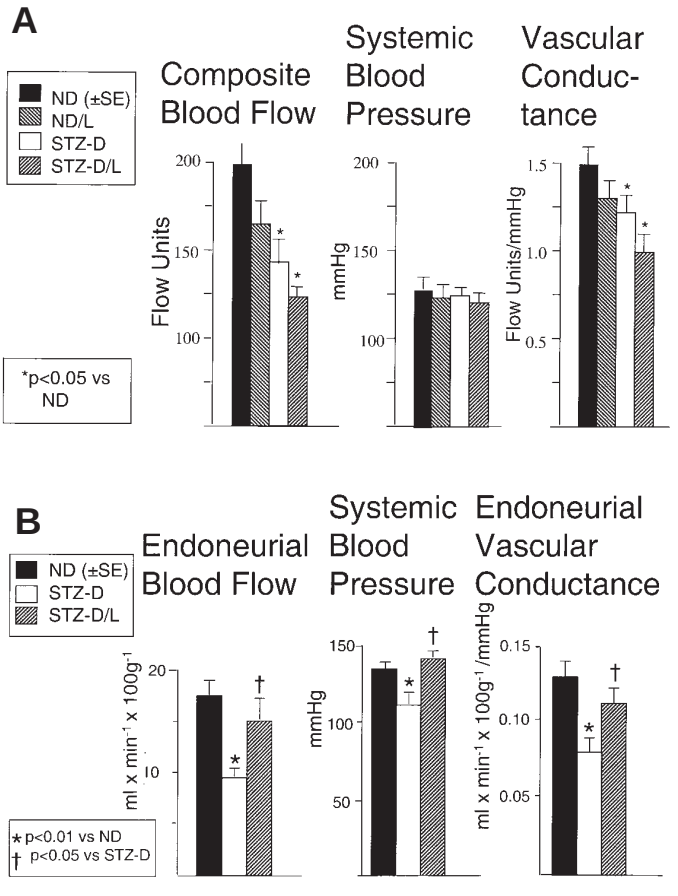

FIG. 2. Effect of STZ-induced diabetes and LA treatment on sciatic NBF and vascular conductance. In experiment 1 (*A*), composite sciatic NBF was measured by laser Doppler flowmetry. In experiment 2 (*B*), sciatic endoneurial nutritive blood flow was measured by H_2-clearance polarography. In each experiment, vascular conductance was calculated by dividing flow by systemic mean blood pressure.

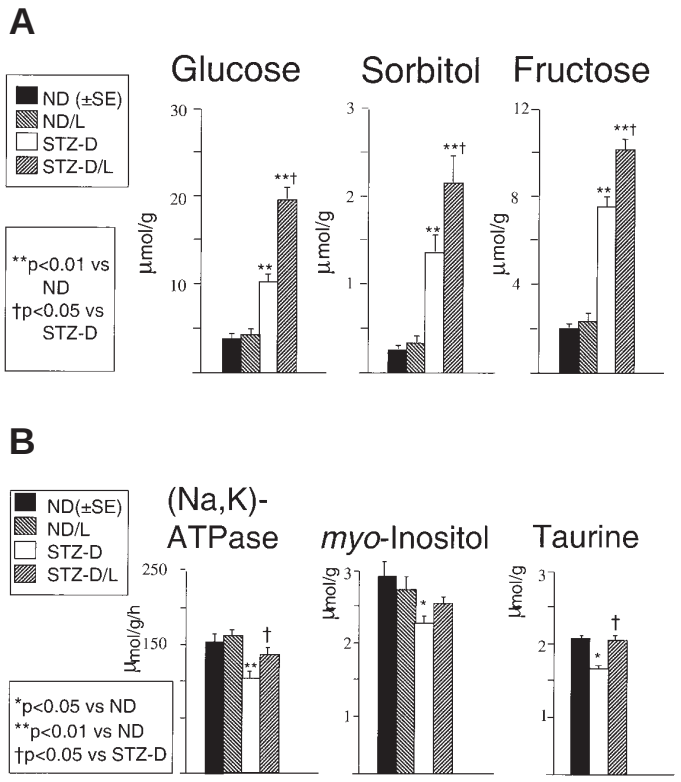

FIG. 3. Effect of STZ-induced diabetes and LA treatment on the content of glucose, sorbitol, fructose, *myo*-inositol, and taurine and on the activity of (Na,K)-ATPase in sciatic nerve. Values are expressed as per gram wet weight of nerve tissue.

TABLE 3
Measurements of metabolic indicators of sciatic nerve energy state

	ND	ND/L	STZ-D	STZ-D/L
n	8	8	8	8
Acetoacetate	0.074 ± 0.007	0.088 ± 0.009	0.531 ± 0.087*†	0.202 ± 0.036‡
β-HB	0.061 ± 0.004	0.072 ± 0.004	1.221 ± 0.190*†	0.236 ± 0.051‡
α-KG	0.136 ± 0.018	0.135 ± 0.007	0.121 ± 0.005	0.141 ± 0.011
Glutamate	1.65 ± 0.06	1.60 ± 0.07	1.82 ± 0.04	1.50 ± 0.06‡
Ammonia	1.40 ± 0.09	1.44 ± 0.13	1.40 ± 0.09	1.40 ± 0.07
Lactate	1.49 ± 0.14	1.54 ± 0.11	2.25 ± 0.16*†	1.98 ± 0.12
Pyruvate	0.167 ± 0.006	0.174 ± 0.009	0.152 ± 0.008	0.189 ± 0.012
Malate	0.118 ± 0.011	0.116 ± 0.007	0.113 ± 0.009	0.099 ± 0.005
3-PG	0.164 ± 0.010	0.155 ± 0.011	0.177 ± 0.007	0.159 ± 0.012
DHAP	0.094 ± 0.006	0.080 ± 0.006	0.118 ± 0.004†	0.102 ± 0.006
ATP	0.902 ± 0.076	0.836 ± 0.060	0.973 ± 0.092	0.904 ± 0.075
ADP	0.388 ± 0.029	0.438 ± 0.049	0.687 ± 0.049*†	0.615 ± 0.022*†
PCr	2.36 ± 0.12	2.41 ± 0.13	1.55 ± 0.13*†	1.56 ± 0.11*†
Cr	4.77 ± 0.35	5.00 ± 0.19	6.18 ± 0.36	6.32 ± 0.37

Data are *n* or means ± SD and are given are given in nanomoles per milligram protein. *P < 0.01 vs. ND; †P < 0.01 vs. ND/L; ‡P < 0.01 vs. STZ-D.

(Fig. 3*B*). Thus, despite exaggerated accumulation of glucose and AR-pathway metabolites, LA administration to STZ-D rats tended to restore a major pathway of energy consumption in peripheral nerve (44) thought to be particularly important to small nerve fiber conduction (45).

Effects of STZ-induced diabetes and LA on nerve energy metabolism. Non–protein-bound metabolically active pools of NAD(P) equilibrate freely with near-equilibrium enzymatic reactions within their subcellular compartments; the equilibrium states of these NAD-linked reactions can be used to estimate the free NAD^+:NADH ratio and the high-energy phosphate potential within specific metabolic compartments. Measurements of individual indicator metabolites in sciatic nerve used to calculate NAD^+:NADH ratios in the mitochondrial and cytoplasmic compartments and measurements of high-energy phosphate compounds are given in Table 3. Levels of acetoacetate and β-HB were unaffected by LA in ND rats, but they were elevated in untreated STZ-D rats and were significantly reduced by LA. Glutamate levels also tended to be higher in untreated STZ-D rats, and they were significantly reduced by LA. Lactate was increased in untreated STZ-D rats versus both ND and ND/L rats (with a similar trend in LA-treated STZ-D [STZ-D/L] rats versus ND and ND/L rats, $0.01 < P < 0.05$). The concentration of DHAP was higher in untreated STZ-D rats versus ND/L rats. ADP levels were higher and PCr levels were lower in untreated STZ-D and STZ-D/L versus ND and ND/L rats, and they were unchanged by LA.

Limited oxygen availability in the ischemic endoneurium in STZ-D has been predicted to limit mitochondrial oxidation of NADH by the respiratory chain (14), which should reduce the free NAD^+:NADH ratio most prominently in the mitochondria. LA administration to ND rats did not alter NAD^+:NADH in mitochondria, whether estimated by the β-HB or glutamate dehydrogenase systems (Fig. 4). Mitochondrial NAD^+:NADH was decreased by ~50% in untreated STZ-D versus ND rats, and this decrease was completely corrected by LA (Fig. 4). Cytosolic NAD^+:NADH was also decreased in untreated STZ-D rats and was corrected by LA administration to levels not significantly different from ND or ND/L rats (Fig. 4).

Cytoplasmic high-energy phosphate stores, whether assessed by ratios of ATP:ADP or PCr:Cr, were decreased in untreated STZ-D rats versus ND or ND/L rats and were unaffected by LA administration in ND rats (Fig. 4). In ND, STZ-D, STZ-D/L, and ND/L rats, respectively, ATP:ADP ratios were 2.37 ± 0.56, 1.46 ± 0.45 (P < 0.05 vs. ND rats), 1.49 ± 0.38 (P < 0.05 vs. ND rats), and 2.08 ± 0.78, and PCr:Cr ratios were 0.520 ± 0.151, 0.257 ± 0.078 (P < 0.01 vs. ND rats), 0.249 ± 0.055 (P < 0.01 vs. ND rats), and 0.485 ± 0.082; absolute values are listed in Table 3. (The published report of increased rather than decreased nerve high-energy phosphate compounds in STZ-D rats [46] may be explained by the use of slow-acting systemic anesthesia for such tissue sampling, since increased metabolic

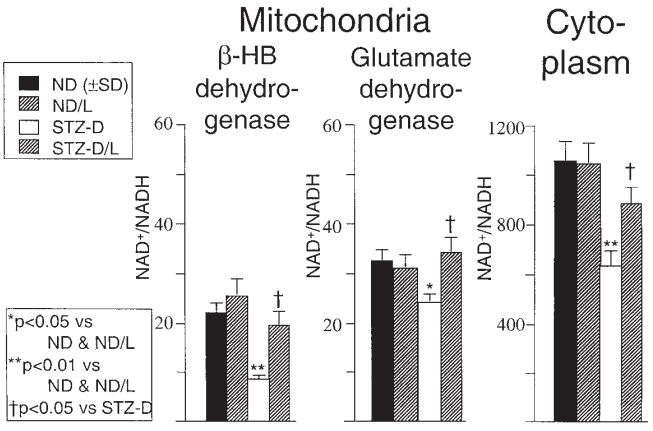

FIG. 4. Effect of STZ-induced diabetes and LA treatment on calculated NAD^+:NADH ratios in the mitochondria and in the cytoplasm. Each NAD^+:NADH ratio was derived and calculated from the measured ratios of metabolites that were in equilibrium with NAD^+ and NADH in each subcellular compartment (β-HB and glutamate dehydrogenase systems for the mitochondria and lactate dehydrogenase system for the cytoplasm), as described in RESEARCH DESIGN AND METHODS. The measured values of the individual metabolites from which these ratios were derived are shown in Table 3.

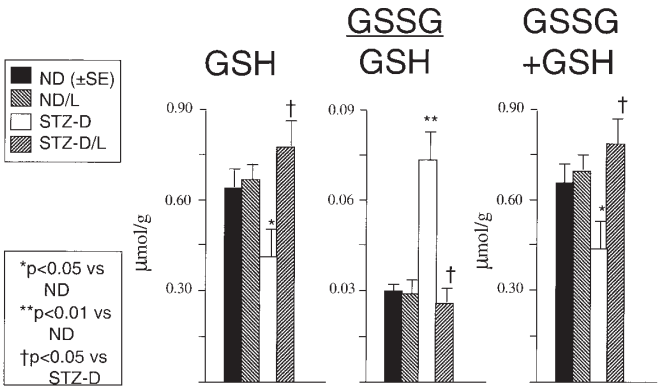

FIG. 5. Effect of STZ-induced diabetes and LA treatment on the content of GSH and GSSG in sciatic nerve. Note the 10-fold difference in the scale of the vertical axes in the center panel versus the left and right panels, indicating that ~90% of total glutathione was in the GSH form.

substrate for and adaptation to anaerobic metabolism may render diabetic nerve tissue more resistant to the bioenergetic effects of anesthetic hypoxia and/or ischemia [47].) Despite the complete correction of endoneurial nutritive NBF and mitochondrial and cytoplasmic NAD^+:NADH ratios by LA treatment of STZ-D rats, cytoplasmic high-energy phosphates remained low, despite LA administration to STZ-D/L rats (Fig. 4).

Effects of STZ-induced diabetes and LA on nerve antioxidants. LA, a well-established antioxidant (27), has been reported to restore the "reduction in GSH associated with a corresponding increase in GSSG" in diabetic rat sciatic nerve (8). In the present experiment, 97% of total nerve glutathione (GSSG + GSH) was in the GSH form in ND rats (Fig. 5). Neither STZ-D nor LA treatment altered the content of GSSG in sciatic nerve (0.019 ± 0.003, 0.022 ± 0.006, 0.025 ± 0.007, and 0.023 ± 0.006 µmol/g in ND, ND/L, STZ-D, and STZ-D/L rats, respectively). LA treatment of ND rats did not alter the sciatic nerve content of GSH or the GSSG:GSH ratio (Fig. 5). However, nerve GSH and GSSG + GSH levels were 50% reduced in untreated STZ-D rats versus ND rats, and the GSSG:GSH ratio was more than doubled (Fig. 5). The reductions in GSH and GSSG + GSH levels and the increase in the GSSG:GSH ratio in STZ-D rats were completed corrected by LA treatment (Fig. 5). Thus, the LA-sensitive glutathione defect in STZ-D rats appears to reflect primarily overall depletion rather than a stoichiometric shift between the oxidized and reduced forms. The content of ascorbate, another endogenous source of reducing equivalents, was not statistically significantly affected by STZ-induced diabetes or LA treatment, although a marginal reduction in untreated STZ-D rats tended to be partially restored by LA treatment: 0.451 ± 0.105, 0.439 ± 0.136, 0.320 ± 0.049, and 0.386 ± 0.098 µmol/g in ND, ND/L, STZ-D, and STZ-D/L rats, respectively (NS).

Effects of STZ-induced diabetes and LA on enzymes of oxidative defense. Key enzyme systems that contribute to tissue defense against oxidative stress are themselves affected by oxidative stress (48) and may be regulated by neurotrophic factors in the nervous system (25). Therefore, the effects of STZ-D and LA on nerve SOD, catalase, GSH transferase, GSSG reductase, and total quinone reductase were assessed (Table 4). No effects of LA treatment on enzymatic activities were observed in ND rats. Activities of both SOD and catalase tended to be decreased in untreated STZ-D rats versus ND rats by 28 and 24%, respectively, although these changes did not attain statistical significance. Conversely, GSHTrans activity was increased by 46%, whereas no difference was found in GSSGRed activities between ND and STZ-D groups. LA treatment completely prevented the diabetes-induced decrease of both SOD and catalase activities with enzyme activities increasing above ND control values. The elevated GSH transferase and GSSG reductase activity levels in STZ-D rats were unaffected by LA. A trend for nerve total quinone reductase activity to be decreased in STZ-D rats and corrected by LA appeared to reside completely in a marked and highly significant decrement in cytochrome b_5 reductase activity, which was completely corrected by LA (Table 4).

DISCUSSION

Abnormalities in nerve energy metabolism, oxidative stress, intracellular signaling, or related metabolic parameters are thought to link hyperglycemia to changes in nerve perfusion and function in experimental DPN. Both free radical-derived oxidative stress ascribed to hyperglycemia (1–8,10,11,13) and decreased endoneurial NBF (1,6,8,13) with ischemic hypoxia (14) have been invoked as primary factors in the pathogenesis of experimental DPN. Decreased NBF may be consequent to free radical-induced vascular dysfunction (1,56); conversely, endoneurial ischemia may precipitate ROS-related damage in peripheral nerve tissue (1). Impaired NBF may reduce NCV directly through nerve energy depletion (1,14) or indirectly through oxidative stress or other secondary metabolic derangements in conducting nerve fibers

TABLE 4
Measurements of oxidative defense enzymes in sciatic nerve

	ND	ND/L	STZ-D	STZ-D/L
n	9	5	8	8
SOD	96.0 ± 6.3	116.2 ± 10.7	70.5 ± 3.5*	136.8 ± 19.7†
Catalase	109.7 ± 9.5	97.5 ± 6.2	77.4 ± 7.0	122.9 ± 12.9‡
GSH transferase	39.2 ± 5.3	49.7 ± 7.2	57.7 ± 4.5	58.0 ± 9.7
GSSG reductase	9.2 ± 1.2	11.3 ± 0.6	10.5 ± 1.1	11.9 ± 1.5
Total quinone reductase	175 ± 11	157 ± 10	131 ± 12	170 ± 16
NADH diaphorase	120 ± 10	122 ± 13	124 ± 11	120 ± 11
Cytochrome b_5 reductase	55.3 ± 2.1†	48.2 ± 4.9	7.4 ± 0.8	50.8 ± 5.6†

Data are *n* or means ± SD and are given in nanomoles per milligram protein. *P < 0.05 vs. ND/L; †P < 0.01 vs. STZ-D; ‡P < 0.05 vs. STZ-D.

(1). This report uses LA, an antioxidant and free radical scavenger, to study the relationship between these critical biochemical parameters and to study specific measures of nerve perfusion and conduction.

LA significantly improved or normalized digital SNCV, endoneurial nutritive NBF, mitochondrial and cytoplasmic NAD^+:NADH ratios, (Na,K)-ATPase activity, GSH and GSH + GSSG content, the GSSG:GSH ratio, and the activities of SOD, catalase, and cytochrome b_5 reductase in STZ-D rats. LA did not improve the decreased sciatic-tibial MNCV, composite (laser Doppler) NBF, or the PCr:Cr and ATP:ADP ratios. LA exaggerated nerve glucose, sorbitol, and fructose accumulation, but ameliorated deficits in nerve *myo*-inositol and taurine.

Divergent effects of LA on NCV, NBF, nerve energy metabolism, nerve osmolytes, and oxidative defense were not entirely unanticipated. Improvement of distal digital SNCV but not sciatic-tibial MNCV, despite correction of endoneurial NBF (8,29), was confirmatory (8), and similar fiber-selective effects of metabolic therapy on NCV are well known in STZ-D rats (49). (The reported improvement of both MNCV and SNCV by LA used different nerve fibers [motor conduction in sciatic nerve fibers innervating the tibialis anterior muscle, and SNCV in the saphenous nerve], older STZ-D rats, and a reversal rather than a prevention paradigm [29].) The consistently divergent (8) preventive effects of LA on sciatic-tibial MNCV and distal digital SNCV in young STZ-D rats could reflect *1*) persistent underperfusion of the specific endoneurial microcompartment containing the sciatic-tibial motor fibers, *2*) selectively enhanced sensitivity of sciatic-tibial motor fibers to residual ischemia (8), *3*) a significant nonvascular component to diabetes-induced MNCV slowing in sciatic-tibial motor fibers (49), and *4*) a direct specific nonvascular action of LA to slow sciatic-tibial MNCV. Persistently diminished (laser Doppler) NBF implies residual LA-resistant regional underperfusion, most likely in the epineurial and perineurial shunt vessels and/or the vessels of the subperineurial space (1). Persistent deficits in sciatic nerve ATP:ADP and PCr:Cr ratios, despite correction of endoneurial nutritive NBF and mitochondrial and cytoplasmic NAD^+:NADH ratios, could be explained by residual localized hypoperfusion, by uncoupling of mitochondrial oxidative phosphorylation, and/or by increased nerve high-energy phosphate consumption. Similarly, exaggeration of nerve glucose, sorbitol, and fructose accumulation, presumably by enhanced glucose uptake (4) with paradoxical (61) amelioration of nerve *myo*-inositol (4,49) and taurine depletion (14), and correction of nerve (Na,K)-ATPase activity (16) could explain the discrepant MNCV and SNCV response to LA if glucose, sorbitol, and fructose were tied to motor fiber function and if *myo*-inositol, taurine, and (Na,K)-ATPase were tied to sensory fiber function. This conforms with the concept that nerve (Na,K)-ATPase resides primarily in smaller myelinated and unmyelinated nerve fibers (50) that predominantly influence SNCV. Amelioration of nerve *myo*-inositol and taurine depletion could reflect restoration by the (Na,K)-ATPase of the Na^+ gradient required for Na^+-dependent uptake. Activation of (Na,K)-ATPase, a major consumer of ATP in nerve tissue (45), could explain persisting decrement in nerve ATP:ADP and PCr:Cr ratios.

The GSH depletion in STZ-D rat nerve was not accompanied by a corresponding increase in GSSG (8) since total glutathione (GSH + GSSG) also declined, excluding the putative AR-related $NADP^+$:NADPH-linked shift in the glutathione reductase reaction (1,26) as the sole explanation of GSH depletion in diabetic nerve. Restoration of sciatic nerve GSH + GSSG but not ATP:ADP and PCr:Cr by LA argues against ATP as rate-limiting for glutathione biosynthesis in STZ-D rats. (The reciprocal changes in sciatic nerve glutamate and GSH produced by LA in STZ-D but not ND rats are consistent with stimulation of flux through γ-glutamylcysteine synthase and/or glutathione synthase.) Whether ATP-dependent transport systems for glutathione adducts, including GSSG, across the plasma membrane (51) and/or transport of GSH into mitochondria (52,53) are affected by STZ-D or LA in nerve remain unexplored (1).

Glutathione may be paramount to the antioxidative defense of nerve, since GSH levels are ~10-fold lower than those in brain and ~6-fold lower than those in lens. The low GSH peroxidase versus catalase activity in peripheral nerve (68) might suggest a minor role for GSH in overall inactivation of H_2O_2. However, H_2O_2 generated by mitochondrial electron transport (52) requires GSH peroxidase inactivation because mitochondria lack catalase (except in the heart [54]). Failure of LA to correct ATP:ADP and PCr:Cr ratios, despite correction of endoneurial NBF, mitochondrial NAD^+:NADH, and GSH content, eliminates overall GSH depletion as the sole basis for mitochondrial damage and/or uncoupling of oxidative phosphorylation in diabetic nerve. However, the mitochondrial GSH pool (15–30% of total [55]) most relevant to ROS generation and mitochondrial oxidative stress (52,53) was not directly measured. Nerve taurine, an important endogenous metal chelator with antioxidant (1,9) and anti–protein kinase C properties (56), was also depleted by STZ-induced diabetes and restored by LA. Localization within microvessels (57) positions taurine depletion to mediate ROS-induced endothelial dysfunction and vasoconstriction (58) in diabetic nerve. Nerve ascorbate, SOD, and catalase deficits in STZ-D rat nerve (10) were improved by LA, recapitulating the LA treatment response of ND rat lens rendered GSH deficient by buthionine sulfoxime (59), thus extending the notion that GSH depletion impairs antioxidative defense enzymes (21), creating a positive feed-forward system of oxidative stress. This could be linked to impaired neurotrophic support since LA corrects deficits in NGF-dependent neuropeptide-Y and substance-P expression in STZ-D rats (2) and because neuronal catalase and SOD activities are regulated by nerve growth factor (NGF) (24,25). The marked cytochrome b_5 reductase deficit in STZ-D rat nerve and its correction by LA are potentially significant. Cytochrome b_5 reductase has been localized to the endoplasmic reticulum (60), the outer mitochondrial membrane (61), and the plasma membrane (62). Moreover, it has been implicated in the maintenance of ascorbate and coenzyme Q in their reduced state and as an alternate pathway for electron transport that importantly contributes to antioxidative defense (62).

In summary, widespread alterations in nerve physiology and biochemistry in 6-week STZ-D rats are consistent with the hypothesis that nerve ischemia, possibly due to oxidative stress, slows some components of MNCV and SNCV by limiting mitochondrial oxygenation and high-energy phosphate availability (26). However, treatment with LA selectively corrected endoneurial nutritive NBF, SNCV, mitochondrial NAD^+:NADH ratios, (Na,K)-ATPase activity, GSH depletion, and some antioxidative defense enzyme activities, but it did not improve sciatic-tibial MNCV or nerve high-energy phos-

phate stores, and it exaggerated accumulation of AR pathway intermediates without further depleting *myo*-inositol and taurine. Thus, complex interactions between defects in nerve biochemistry and in nerve perfusion produced by STZ-induced diabetes and corrected by LA appear to impact differentially on different nerve fiber populations (1). These findings are consistent with the recent clinical observations that treatment with LA (43) or NGF appears to improve sensory parameters without improving MNCV in human DPN.

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