α-Lipoic Acid: Effect on Glucose Uptake, Sorbitol Pathway, and Energy Metabolism in Experimental Diabetic Neuropathy

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The peripheral nerve of experimental diabetic neuropathy (EDN) is reported to be ischemic and hypoxic, with an increased dependence on anaerobic metabolism, requiring increased energy substrate stores. When glucose stores become reduced, fiber degeneration has been reported. We evaluated glucose uptake, nerve energy metabolism, the polyol pathway, and protein kinase C (PKC) activity in EDN induced by streptozotocin. Control and diabetic rats received lipoic acid (0, 10, 25, 50, 100 mg/kg). Duration of diabetes was 1 month, and α-lipoic acid was administered intraperitoneally 5 times per week for the final week of the experiment. Nerve glucose uptake was reduced to 60, 37, and 30% of control values in the sciatic nerve, L5 dorsal root ganglion, and superior cervical ganglion (SCG), respectively, in rats with EDN. α-Lipoic acid supplementation had no effect on glucose uptake in normal nerves at any dose, but reversed the deficit in EDN, with a threshold between 10 and 25 mg/kg. Endoneurial glucose, fructose, sorbitol, and *myo*-inositol were measured in sciatic nerve. α-Lipoic acid had no significant effect on either energy metabolism or polyol pathway of normal nerves. In EDN, endoneurial glucose, fructose, and sorbitol were significantly increased, while *myo*-inositol was significantly reduced. α-Lipoic acid had a biphasic effect: it dose-dependently increased fructose, glucose, and sorbitol, peaking at 25 mg/kg, and then fell beyond that dose, and it dose-dependently increased *myo*-inositol. Sciatic nerve cytosolic PKC was increased in EDN. ATP, creatine phosphate, and lactate were measured in sciatic nerve and SCG. α-Lipoic acid prevented the reduction in SCG creatine phosphate. We conclude that glucose uptake is reduced in EDN and that this deficit is dose-dependently reversed by α-lipoic acid, a change associated with an improvement in peripheral nerve function. *Diabetes* 48:2045–2051, 1999

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ANOVA, analysis of variance; 2-DG, [^{14}C]-2-deoxy-D-glucose; DRG, dorsal root ganglion; EC_{50}, dose corresponding to 50% of the response; EDN, experimental diabetic neuropathy; MAP, mean arterial blood pressure; PKC, protein kinase C; SCG, superior cervical ganglion.

Experimental diabetic neuropathy (EDN), induced by streptozotocin, has a blood flow deficit of 50% and is hypoxic (1–4). Hyperglycemia-induced ischemic and auto-oxidative lipid peroxidation is suggested to cause neuropathy (5). The energy status in EDN is complex. On the one hand, endoneurial glucose is increased (6,7), and the increased energy substrate is protective, allowing the peripheral nerve of EDN to conduct impulses for a longer time than normal nerves when its blood supply or oxygenation is cut off (8). On the other hand, the ischemic and hypoxic peripheral nerve of EDN, which has an increased dependence on inefficient anaerobic metabolism (8,9), is very susceptible to reductions in energy substrates. For instance, insulin treatment, important in correcting insulin deficiency in insulin-sensitive tissue (10), could, by reducing energy substrate stores in non–insulin-sensitive ischemic peripheral nerve, result in an energy crisis and the development of axonal degeneration (11,12), a situation akin to "insulin neuritis" and the temporary worsening of neuropathy that occasionally occurs in human subjects when tight insulin-induced glycemic control is imposed (13–15). Similarly, diabetic peripheral nerve is more liable to undergo ischemic fiber degeneration than normal nerves (16). α-Lipoic acid will increase glucose uptake in both insulin-sensitive and non–insulin-sensitive tissues (17–19). A multicenter study on the efficacy of α-lipoic acid in the treatment of human diabetic neuropathy is underway. Therefore, it is important to study the effect of the drug on glucose uptake, polyol pathway, and energy metabolism. In this study, we evaluated, using ^{14}C-deoxyglucose, whether glucose uptake was altered in EDN and the efficacy of α-lipoic acid in reversing such an abnormality. We also report a dose-response study of the effect of α-lipoic acid treatment on endoneurial glucose, fructose, sorbitol, *myo*-inositol, nerve protein kinase C (PKC) activity, and energy metabolites in diabetic peripheral nerve.

RESEARCH DESIGN AND METHODS

Experimental animals. We used a total of 263 male Sprague-Dawley rats, beginning weight 250 ± 5 g. They were fed Purina Rodent Laboratory Chow (no. 5001; Richmond, IN) with a liberal supply of water and were housed in plastic containers whose floors were covered with wood shavings. The study was done in multiple stages. Stage 1 was a study on glucose uptake. A total of 102 rats were divided into the following groups, with at least seven rats in each group:

Group 1: Controls without α-lipoic acid (C0)
Group 2: Controls + α-lipoic acid 10 mg/kg (C10)
Group 3: Controls + α-lipoic acid 25 mg/kg (C25)

Group 4: Controls + α-lipoic acid 50 mg/kg (C50)
Group 5: Controls + α-lipoic acid 100 mg/kg (C100)
Group 6: Streptozotocin diabetes for 1 month (D0)
Group 7: Streptozotocin diabetes for 1 month + α-lipoic acid 10 mg/kg (D10)
Group 8: Streptozotocin diabetes for 1 month + α-lipoic acid 25 mg/kg (D25)
Group 9: Streptozotocin diabetes for 1 month + α-lipoic acid 50 mg/kg (D50)
Group 10: Streptozotocin diabetes for 1 month + α-lipoic acid 100 mg/kg (D100)

For stage 2 of the study, energy metabolism and the polyol pathway were studied using the following groups. A total of 75 animals were used in this part of the study.

Group 1: Controls without α-lipoic acid (C0)
Group 2: Controls + α-lipoic acid 100 mg/kg (C100)
Group 3: Streptozotocin diabetes for 1 month (D0)
Group 4: Streptozotocin diabetes for 1 month + α-lipoic acid 100 mg/kg (D100)

For stage 3 of the study, the effect of dose of α-lipoic acid on polyol pathway and on sciatic nerve PKC was studied using 86 rats divided into the following groups:

Group 1: Controls without α-lipoic acid (C0)
Group 2: Streptozotocin diabetes for 1 month (D0)
Group 3: Streptozotocin diabetes for 1 month + α-lipoic acid 10 mg/kg (D10)
Group 4: Streptozotocin diabetes for 1 month + α-lipoic acid 25 mg/kg (D25)
Group 5: Streptozotocin diabetes for 1 month + α-lipoic acid 50 mg/kg (D50)
Group 6: Streptozotocin diabetes for 1 month + α-lipoic acid 100 mg/kg (D100)

Streptozotocin and α-lipoic acid. Experimental diabetes was produced by the intraperitoneal injection of streptozotocin in 0.05 mol/l citrate buffer at pH 4.5 (65 mg/ml; dose 1.32 ml/kg). The control group received an intraperitoneal injection of citrate buffer alone (20).

We administered racemic α-lipoic acid, at the concentrations listed above, intraperitoneally daily for five doses during the final week of the experiment. The powder was mixed with saline, 2 mol/l NaOH was added to dissolve the suspension, and then the pH was brought to 7.4 with 2 mol/l HCl. Saline was used to bring the solution to the final concentration.

At 1 month, at the terminal experiment, the animals were fasted overnight, weighed, and anesthetized with pentobarbital sodium (60 mg/kg). Arterial blood was drawn for glucose and glycosylated hemoglobin. For studies of energy metabolism, the sciatic nerve on the left side and the superior cervical ganglion (SCG) were harvested. For studies of the polyol pathway, sciatic nerve and L5 dorsal root ganglia (DRGs) were obtained. For PKC studies, sciatic nerve was obtained.

[^{14}C]-2-Deoxy-D-glucose uptake. At 1 month, at the terminal experiment, the animals were fasted overnight, weighed, and anesthetized with pentobarbital sodium 60 mg/kg. Arterial blood was drawn for glucose and glycosylated hemoglobin testing. Rectal temperature was monitored and maintained between 36.5 and 37.5°C using a thermistor probe connected to a digital thermometer (model BAT 8; Baily Instruments, Saddle Brook, NJ) attached to a control feedback unit (TCAT-1; Physitemp, Clifton, NJ) and an infrared lamp. A tracheostomy tube was inserted, and a polyethylene catheter (PE-50; Becton Dickinson, Sparks, MD) was placed in the right common carotid artery for monitoring of systemic mean arterial blood pressure (MAP) and for measurements of blood gases and pH. The right brachial artery was also exposed, and a catheter (PE-50, tapered tip) was inserted and used to collect blood samples. The right femoral vein was cannulated for administration of the isotope. Artificial ventilation with a mixture of nitrogen and oxygen gases was provided by a rodent respirator (model 683; Harvard Apparatus, Mills, MA). The rats were then paralyzed with D-tubocurarine (1.5–2.0 mg/kg, intra-arterial injection). The adequacy of anesthesia was monitored by the level and stability of the MAP and absence of the corneal reflex, and adequate levels of anesthesia and analgesia were ensured with supplemental intraperitoneal injection of pentobarbital sodium given as required. MAP was measured with a pressure transducer (P23dB; Statham, Hato Rey, Puerto Rico) and recorded on a polygraph chart recorder (model 7D Polygraph; Grass Instruments, Quincy, MA). Arterial blood was sampled every 15 min to ensure that pH and gas values remained within the physiological range (Blood Gas Manager model 1312; Instrumentation Laboratory, Milano, Italy), and was also sampled for determinations of glucose and glycosylated hemoglobin. MAP and arterial blood gas data were maintained within the physiological range throughout all experiments.

We infused [^{14}C]-2-deoxy-D-glucose (2-DG), 16.7 mCi/kg dissolved in physiological saline, via the femoral vein at a constant rate within 30 s. Some 13 arterial samples in duplicate (20 µl each) were withdrawn over the next 45 min at a defined schedule (0, 0.5, 1, 2, 3, 5, 8, 10, 15, 20, 30, 40, 45 min). One sample was analyzed for glucose, and the other was digested with a mixture of 1:2 protosol:ethanol in a shaker bath at 50°C, decolored with 30% hydrogen peroxide, mixed with 10 ml liquid scintillation cocktail (Ultima Gold; Packard Instruments, Meriden, CT), and then counted in a Beckman beta particle counter (model LS5000TD; Beckman Instruments, Fullerton, CA). After 45 min, the rat was killed with an overdose of 2 mol/l KCl. Both sciatic nerves were dissected out, stretch mounted on wax, and snap frozen in isopentane and liquid nitrogen at –70°C. Both left and right L5 DRGs and both SCGs were similarly immediately frozen in liquid nitrogen. The tissues were transferred to a cryostat, and 20 µm sections were cut and apposed to gelatin-subbed slides. These slides and standards were then mounted in apposition to film (Ultrafilm; Eastman Kodak, Rochester, NY) for 37 days in light-proof cassettes. The films were then processed in the dark room with developer and fixative. Autoradiographic processing was performed using the microcomputer imaging device system (MCID; Imaging, St. Catherines, Ontario, Canada). Blood counts (dpm), glucose levels collected at the described time points, and densitometry calibration standards were fitted to an equation for glucose utilization with the Savaki modification of the Sokoloff equation in the MCID. The rate constants and lumped constant for white (and gray) matter are available (21). A color map of nerve glucose utilization was displayed with a calibration bar.

Whole blood glucose and glycosylated hemoglobin. Whole blood glucose was determined using a glucose oxidase method. Measurements were done in duplicate at 450 nm on a Beckman DU-7400 Spectrophotometer (20). Glycosylated hemoglobin was assayed by affinity column as previously described (20,22).

Nerve ATP, creatine phosphate, and lactate. Tissue extract was prepared as previously described (8). In brief, ~17 mg of sciatic nerve or 4 mg of SCG were homogenized in perchloric acid and centrifuged; the supernatant was neutralized in potassium carbonate; and the precipitate was removed by centrifugation. Nerve ATP, creatine phosphate, and lactate estimations were made in duplicate on aliquots of supernate using the fluorometric method of Lowry and Passoneau (23).

Nerve glucose, fructose, sorbitol, and *myo*-inositol. Nerve glucose, fructose, sorbitol, and *myo*-inositol were determined using gas chromatography as previously detailed (24). In brief, ~2 cm of desheathed sciatic nerve or L5 DRGs were weighed, lyophilized, and homogenized, and trimethylsilyl O-methyloximes of carbohydrates were prepared (25). The trimethylsilyl derivatives were quantitated using a Hewlett-Packard capillary gas chromatograph (Palo Alto, CA).

PKC activity. Sciatic nerve was harvested, immediately frozen in liquid N_2, and stored at –80°C. All tissue preparation steps were performed at 4°C or on ice and were based on the method of Wakasaki et al. (26). At the time of assay, tissues were minced and homogenized in buffer A (20 mmol/l Tris-HCl, pH 7.5; 2 mmol/l EDTA; 0.5 mmol/l EGTA; 0.057 mmol/l phenylmethylsulfonyl fluoride; 25 µg/ml leupeptin; 0.1 mg/ml aprotinin; 0.33 mol/l sucrose) using a Dounce homogenizer (Fisher Scientific, Pittsburgh, PA; 50 strokes loose pestle, 50 strokes tight pestle). The homogenates were centrifuged at 1,000*g* for 10 min, and the resulting supernatant at 100,000*g* for 30 min. The subsequent supernatant was processed as the cytosol fraction. Pellets were resuspended in buffer B (buffer A without sucrose), solubilized in 1% Triton X-100 for 45 min, and centrifuged at 100,000*g* as before. This subsequent supernatant was processed as the membrane fraction. Cytosolic and membrane fractions were purified on DEAE Sephacel (Amersham Pharmacia Biotech, Piscataway, NJ) columns and eluted with 200 mmol/l NaCl in buffer B. PKC activities were measured using the Biotrak PKC enzyme assay system (Amersham Life Science, Arlington Heights, IL).

Statistics. For comparison among groups, analysis of variance (ANOVA) was used, with Dunnett's post hoc test used to compare the effect of dose of α-lipoic acid on control and diabetic rats. For a comparison of the effect of α-lipoic acid on diabetic nerves, the two-tailed *t* test for unpaired group data was used. Data are expressed as means ± SE. Significance was accepted when $P < 0.05$. For evaluation of dose-response studies, we undertook nonlinear regression curve fitting. The data fitted a sigmoid curve (four-parameter logistic equation), with a variable Hill coefficient. For the effect of α-lipoic acid on nerve *myo*-inositol, we used linear regression analysis.

RESULTS

Animals. The weights, blood glucose, and glycosylated hemoglobin all showed significant differences among groups ($P < 0.001$; Table 1). All diabetic rats were severely hyperglycemic, lost weight, and had increased glycosylated hemoglobin. The blood glucose and glycosylated hemoglobin of diabetic rats, both unsupplemented (D0) and supplemented (D10, D25, D50, D100), were significantly increased ($P < 0.001$) over those of unsupplemented (C0) and supplemented (C10, C25, C50, C100) control rats. Hyperglycemia was associated with a significantly reduced weight ($P < 0.001$) in all diabetic groups when compared with control groups. α-Lipoic acid supplementation did not affect the weights, blood glucose, or glycosylated hemoglobin of either control or diabetic rats.

TABLE 1
Weight, glucose, and glycosylated hemoglobin values in control rats (C0), rats with EDN (D0), and α-lipoic acid–supplemented rats (C10, C25, C50, C100, D10, D25, D50, D100)

Group	*n*	Weight (g)	Glucose (mg/dl)	HbA_1 (%)
C0	51	377.2 ± 4.7	118.4 ± 4.8	5.0 ± 0.2
C10	8	355.0 ± 10.6	122.3 ± 6.0	3.9 ± 0.1
C25	8	384.4 ± 7.0	117.8 ± 4.2	4.2 ± 0.2
C50	7	354.3 ± 4.1	132.5 ± 4.7	3.7 ± 0.3
C100	29	355.9 ± 5.0	98.0 ± 4.4	4.4 ± 0.1
D0	48	216.7 ± 5.7*	494.0 ± 15.1*	15.5 ± 0.6*
D10	21	213.7 ± 9.2*	501.6 ± 18.2*	14.2 ± 0.4*
D25	20	194.9 ± 10.0*	567.4 ± 23.9*	14.7 ± 0.5*
D50	29	226.2 ± 9.7*	492.0 ± 20.9*	14.7 ± 0.5*
D100	42	231.0 ± 7.4*	438.0 ± 17.6*	15.0 ± 0.3*

Data are means ± SE. *P < 0.001 vs. control, Dunnett's test.

Glucose uptake

Sciatic nerve. Glucose uptake, measured as 2-DG activity, was significantly different among groups (P < 0.001, ANOVA). Autoradiographic density was evenly distributed in the autoradiographs of all tissues (data not shown). Glucose uptake was significantly reduced (P < 0.001, two-tailed t test for unpaired groups) in the sciatic nerve of rats with EDN (10.7 ± 0.6 mmol · 100 g^{-1} · min^{-1}; Fig. 1), compared with a control value of 17.8 ± 0.4 mmol · 100 g^{-1} · min^{-1}. α-Lipoic acid supplementation had no effect on control sciatic nerve (P > 0.05, ANOVA), but corrected the deficit in glucose uptake on EDN sciatic nerve, in a dose-dependent fashion, completely at the highest dose (Fig. 1). The dose-response curve was expressed as log of dose versus uptake:

$$Y = B + (T - B)/(1 + 10^{\log EC_{50} - X \times \text{Hill slope}})$$

where X = log concentration of α-lipoic acid, Y = the 2-DG uptake, beginning at B (bottom values) and following a sigmoid curve to reach T (asymptote [top]), and EC_{50} describes the dose (effective concentration) corresponding to 50% of the response. For sciatic nerve, B = 9.533, T = 17.668, and EC_{50} = 1.454 (log units, converting to 28.5 mg/kg α-lipoic acid).

DRG. Glucose uptake for L5 DRG was significantly reduced (P < 0.001, two-tailed t test for unpaired groups) in EDN (16.9 ± 1.6 mmol · 100 g^{-1} · min^{-1}; Fig. 1), compared with the control value of 45.5 ± 2.3 mmol · 100 g^{-1} · min^{-1}. α-Lipoic acid supplementation had no effect on control DRG at any dose (P > 0.05, ANOVA), but dose-dependently prevented the reduced uptake in EDN completely at 100 mg/kg. The dose-response curve does not achieve an asymptote. We analyzed the data in two ways. First, using EDN data only, log dose versus uptake: B = 16.019, T = 56.413, and EC_{50} = 1.719 (log units, converting to 52.4 mg/kg α-lipoic acid). If we assume that controls (all doses) and D100 represent the correct asymptote, corresponding values were: B = 16.45, T = 43.17, and EC_{50} = 1.599 (log units, converting to 39.69 mg/kg α-lipoic acid).

SCG. Glucose uptake for SCG was significantly reduced (P < 0.001, two-tailed t test for unpaired groups) in EDN (17.0 ± 1.5 mmol · 100 g^{-1} · min^{-1}; Fig. 1), compared with the control value of 55.8 ± 3.7 mmol · 100 g^{-1} · min^{-1}. α-Lipoic acid supplementation had no effect on control SCG (P > 0.05, ANOVA), but dose-dependently prevented the deficit in uptake in EDN. The dose-response curve was expressed as log dose versus uptake: B = 20.192, T = 61.438, and EC_{50} = 1.557 (log units, converting to 36.1 mg/kg α-lipoic acid).

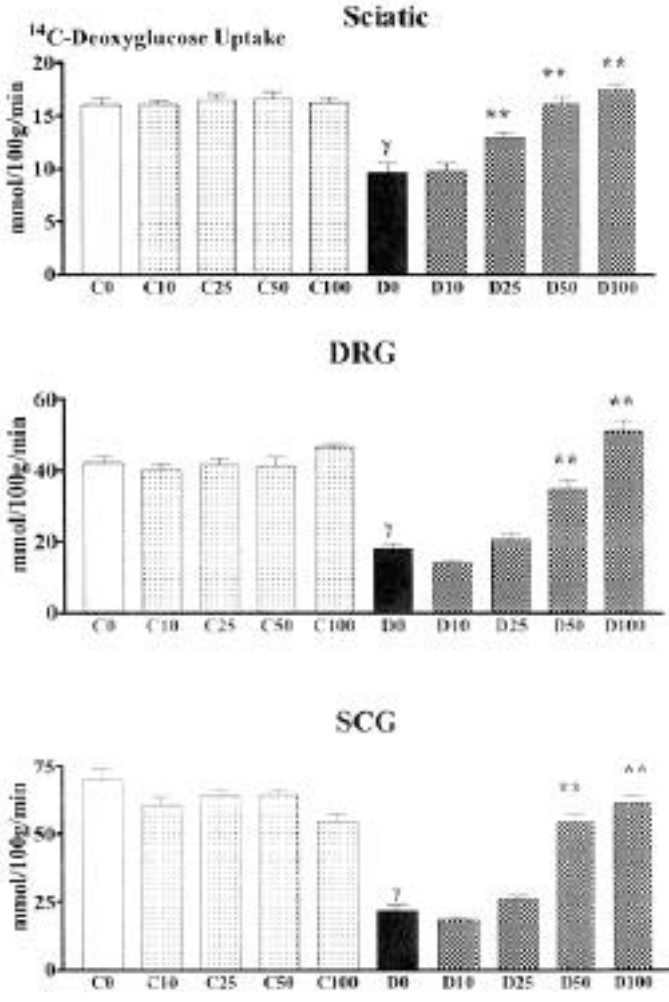

FIG. 1. ^{14}C-deoxyglucose uptake by control (C) and diabetic (D) sciatic nerve, L5 DRG, and SCG in response to α-lipoic acid in doses of 0, 10, 25, 50, and 100 mg · kg^{-1} · day^{-1}. Dose of α-lipoic acid is indicated by the value next to the symbol (i.e., C0 and D25 represent control rats without α-lipoic acid supplementation and diabetic rats with 25 mg · kg^{-1} · day^{-1} supplementation). γP < 0.001 for D0 vs. C0, Dunnett's test; **P < 0.01 for D0 vs. α-lipoic acid–supplemented rats, unpaired two-tailed t test.

Polyol pathway. α-Lipoic acid (100 mg/kg) had no significant effect on fructose, glucose, sorbitol, or *myo*-inositol of control sciatic nerve (data not shown). The diabetic state resulted in a large and significant increase in endoneurial fructose, glucose, and sorbitol and a significant reduction in *myo*-inositol (Table 2). The effects of α-lipoic acid were biphasic. It dose-dependently increased fructose, glucose, and sorbitol to a dose of 25 mg/kg, and then underwent a reduction above this dose. *myo*-Inositol increased with the dose of α-lipoic acid. The relationship between *myo*-inositol and lipoic acid fitted a linear regression Y = 7.92 + 0.02X, where Y = *myo*-inositol (nanomoles per milligram dry weight) and X is dose of α-lipoic acid (milligrams per kilogram), R^2 = 0.76. *myo*-Inositol values of treated diabetic nerves were significantly higher than untreated (D0) diabetic nerves (Table 2).

TABLE 2
Effect of α-lipoic acid on sciatic nerve fructose, glucose, sorbitol, and *myo*-inositol in control (C0), EDN (D0), and α-lipoic acid–supplemented (D10, D25, D50, D100) rats

Group	Nerves (*n*)	Fructose	Glucose	Sorbitol	*myo*-Inositol
C0	12	2.85 ± 0.20	3.94 ± 0.38	1.31 ± 0.29	14.37 ± 0.64
D0	8	15.54 ± 0.92*	13.77 ± 1.69*	7.46 ± 0.69*	7.8 ± 0.22*
D10	8	17.68 ± 1.18*	13.70 ± 1.35*	12.23 ± 1.47*	7.57 ± 0.28*
D25	9	24.65 ± 2.36*	23.08 ± 4.03*	17.65 ± 2.99*	9.08 ± 0.21*†
D50	14	13.96 ± 1.03*	8.83 ± 0.79	7.60 ± 0.82*	9.02 ± 0.40*‡
D100	5	15.63 ± 0.39*	10.06 ± 0.48	7.75 ± 0.52*	9.68 ± 0.36*†

Data are means ± SE and are expressed as nanomoles per milligram dry weight. *$P < 0.001$ vs. C0, Dunnett's test; †$P < 0.001$, ‡$P < 0.05$ vs. D0, unpaired *t* test.

PKC. The cytosolic PKC activity in sciatic nerve is three- to fivefold higher than that of membrane ($P < 0.001$). Cytosolic PKC was significantly increased in sciatic nerve of rats with EDN compared with controls (Table 3). Levels of PKC activity in the membrane fraction paralleled the cytosolic fraction. These changes (cytosolic and membrane) approximately mirrored the changes in levels of sugar alcohols. In the sciatic nerves of α-lipoic acid–supplemented rats, the dose-dependent increase in sugar alcohols (at doses of α-lipoic acid up to 25 mg/kg) was unassociated with any further increase in PKC activity. Instead, there was a modest nonsignificant reduction in PKC activity.

Energy metabolism. ATP, creatine phosphate, and lactate levels for sciatic nerve and SCG are shown in Table 4. Sciatic nerve shows a significant increase in lactate in rats with EDN compared with controls ($P < 0.01$). In SCG, creatine phosphate is reduced ($P < 0.05$), and α-lipoic acid treatment significantly increased the creatine phosphate of EDN ($P < 0.001$), returning creatine phosphate values to control values.

DISCUSSION

There are several key findings of this study. First, glucose uptake is significantly reduced ($P < 0.001$) to 60, 37, and 30% of control values in the sciatic nerve, L5 DRG, and SCG, respectively, in EDN. Second, α-lipoic acid dose-dependently corrects the deficit in glucose uptake into peripheral nerve of diabetic, but not normal, nerve. This finding is consistent in both nerve trunk and cell bodies (sciatic nerve, L5 DRG, SCG) studied. The diabetic state resulted in a significant increase in glucose, fructose, and sorbitol, and a reduction in *myo*-inositol, as previously described (27). α-Lipoic acid, up to a dose of 25 mg/kg, dose-dependently increased endoneurial glucose, fructose, and sorbitol, but these sugars fell at higher doses of α-lipoic acid. α-Lipoic acid dose-dependently increases endoneurial *myo*-inositol in EDN.

TABLE 3
Sciatic nerve PKC in control (C0), EDN (D0), and α-lipoic acid–supplemented (D10, D25, D50, D100) rats

		Sciatic nerve	
Group	Nerves (*n*)	Cytosol	Membrane
C0	11	15,789 ± 1,144	3,864 ± 648
D0	11	24,331 ± 2,333*	5,876 ± 956
D10	11	23,455 ± 1,527*	6,438 ± 1,358
D25	10	24,949 ± 1,795*	8,756 ± 1,590†
D50	14	21,745 ± 1,305†	6,083 ± 787
D100	8	21,835 ± 1,406	5,871 ± 1,132

Data are means ± SE and are expressed as picomoles per milligram per minute. *$P < 0.01$, †$P < 0.05$ vs. C0, Dunnett's test.

We measured glucose uptake using the Savaki (28) modification of the Sokoloff method (21), using 2-DG as the label. This molecule is transported across tight junctions (blood-nerve and perineurial barriers) by GLUT1 (29–31) and is phosphorylated by hexokinase to 2-DG-6-phosphate, but is not metabolized further (21). The autoradiographic concentration of trapped 2-DG-phosphate reflects glucose-6-phosphate and, hence, the rate of combined glucose phosphorylation and transport. By relating the activity of 2-DG in neural tissue to the time course of arterial concentrations of glucose and 2-DG, and using the lumped constant (which describes the kinetic behavior of 2-DG and glucose) (21,32), glucose uptake (transport and phosphorylation) can be accurately determined. Lumped constants for peripheral nerve have not been determined (33), but separate constants for brain white and gray matter are available (21). Sources of error have been discussed for brain (21,28) and nerve (33). The choice of the particular lumped constant does not affect the statistical analyses we made, since the focus was on comparisons between control and diabetic tissues, and between untreated and treated diabetic tissues, where the relevant constant is identical for the groups compared. It could, however, affect the absolute values. The selection of lumped constants for brain white matter for nerve and brain gray matter for DRG and SCG are likely to be similar.

Whether hyperglycemia reduces glucose uptake by an effect on transport (GLUT1) or metabolism (hexokinase) or by reducing tissue perfusion (34–36) is uncertain. The erythroid glucose transporter (GLUT1) is localized in the microvessels of sciatic nerve and DRG and in the perineurium of sciatic nerve (29–31). GLUT1 is also readily demonstrable in human sciatic nerve (37). In contrast, GLUT3 is either negligible or absent in human and rat sciatic nerve (29,31,37–39). Hyperglycemia reduces gene expression of GLUT1 in diabetic rat brain (19,39–41). However, there are also reports of no change (17,41,42) or an increase (43). The diabetic state does not qualitatively cause any immunocytochemical changes in GLUT1 expression in diabetic nerves (38). The activity of nerve hexokinase, which phosphorylates glucose (44,45), is unknown. We did not find a significant reduction of enzymatic activity in experimental hypoxic neuropathy (9).

The three tissues (sciatic nerve, DRG, and SCG) differ in their tight junction and blood flow. Sciatic nerve has both a

TABLE 4
ATP, creatine phosphate, and lactate levels for sciatic nerve and SCG in control (C0), EDN (D0), and α-lipoic acid–supplemented (C100, D100) rats

Group	n	Sciatic nerve			SCG		
		ATP	Creatine phosphate	Lactate	ATP	Creatine phosphate	Lactate
C0	10	0.82 ± 0.02	1.74 ± 0.04	0.78 ± 0.05	1.49 ± 0.09	1.38 ± 0.13	1.50 ± 0.09
C100	9	0.79 ± 0.02	1.80 ± 0.05	0.84 ± 0.06	1.38 ± 0.11	1.46 ± 0.19	1.56 ± 0.12
D0	9	0.99 ± 0.05*	1.64 ± 0.10	1.68 ± 0.13*	1.33 ± 0.06	0.85 ± 0.10†	1.96 ± 0.24
D100	9	0.87 ± 0.03	1.52 ± 0.08	1.31 ± 0.10‡	1.56 ± 0.10	1.47 ± 0.07§	2.41 ± 0.30*

Data are means ± SE and are expressed as millimoles per kilogram wet weight. *P < 0.01; †P < 0.05 vs. C0, Dunnett's test; ‡P < 0.05; §P < 0.001 for D0 vs. D100, unpaired two-tailed t test.

perineurial and blood-nerve barrier; DRG and SCG have much less secure blood-nerve barriers (46,47). Sciatic nerve, DRG, and SCG rank from lowest to highest in both metabolic activity and blood flow (34). Glucose uptake for diabetic sciatic nerve, DRG, and SCG are reduced to 60, 37, and 30% of control, respectively, an order that is more concordant with metabolic activity and blood flow than GLUT1 activity (34), lending support to the notion that α-lipoic acid normalizes the deficit in glucose uptake by normalizing neural tissue blood flow rather than transport, which appears to be independent of glucose concentration in the medium (48). Indeed, the EC_{50} for the effects of α-lipoic acid on glucose uptake (28.5 mg/kg) and on nerve blood flow (calculated from the published values of Nagamatsu et al. [35], ~30 mg/kg) are similar.

α-Lipoic acid increases glucose availability in both insulin-sensitive and insulin-insensitive tissues (49–53). Irrespective of the mechanism of improvement in glucose uptake, a key question is whether this increase is beneficial or deleterious. The normalization of glucose uptake on glycolysis, or its entry into the Kreb's tricarboxylic acid cycle, could improve ATP production, and be beneficial to diabetic nerve, especially under conditions of increased energy demand, or when blood and nerve glucose are reduced (as with insulin treatment). Mechanisms of α-lipoic acid downstream of hexokinase that might facilitate glycolysis include its role as a redox couple (lipoic:dihydrolipoic acid) normalizing NADH and its role facilitating entry into the Krebs cycle. There is evidence, in certain tissues, for a coupling of increased glucose entry with improved energy stores (49,52,54). These findings were made in rat heart (52) and in human diabetic muscle due to diabetes (54,55) and mitochondrial cytopathy (49). Our findings of dose-dependent normalization of glucose uptake in diabetic peripheral nerve tissue have not been previously reported.

The action of α-lipoic acid on glucose uptake in EDN appears to be a specific action because it corrects the deficit in EDN but has no effect on normal nerves. In hypoxic neural tissue, with an increased dependence on anaerobic metabolism, increased endoneurial glucose stores would improve energy metabolism under conditions of increased energy demand. Indeed, hypoxic nerve has been reported to continue to function on glucose alone in a completely anoxic environment (56). A potential detrimental effect is hyperactivity of the polyol pathway, mediated by aldose reductase and sorbitol dehydrogenase, resulting in an increase in sorbitol and a reduction in *myo*-inositol (57). The latter reduction has been hypothesized to cause neuropathy via alteration of polyphosphoinositide metabolism, resulting in a reduction in Na^+-K^+-ATPase activity (57). The mechanisms by which hyperactivity of the polyol pathway reduces *myo*-inositol is not fully known, although hyperglycemia could reduce nerve *myo*-inositol by competitive inhibition of peripheral nerve *myo*-inositol uptake (58). Osmolyte and nonosmolyte mechanisms could be involved (59). A "compatible osmolyte hypothesis" (60,61) proposes that intracellular nonionic organic osmolytes, such as sorbitol, *myo*-inositol, taurine, betaine, and glycerophosphorylcholine, respond coordinately to changes in external osmolality, thereby maintaining the intracellular ionic milieu. Glucose-induced sorbitol accumulation in isosmotic hyperglycemic states is associated with compensatory depletion of *myo*-inositol and taurine (61). The increase in sorbitol and fructose, secondary to α-lipoic acid–mediated increase in glucose uptake, would be expected to further lower *myo*-inositol levels via the osmolyte mechanism (60,61). Presumably, α-lipoic acid increases *myo*-inositol by yet unknown nonosmolyte mechanisms. Sorbitol per se is nontoxic (62), and it is likely that it is mechanisms other than nerve sorbitol accumulation that cause neuropathy. In retinal microvessels, hyperglycemia has been proposed to cause hypoxia by increasing activity of PKC-reducing Na^+-K^+-ATPase activity (63). *myo*-Inositol reduction is associated with reduced Na^+-K^+-ATPase activity (7). α-Lipoic acid normalizes endoneurial Na^+-K^+-ATPase activity of experimental diabetic nerves (D. Greene, personal communication; N. Cameron, personal communication). The improved Na^+-K^+-ATPase activity could improve *myo*-inositol uptake by the Na^+-*myo*-inositol cotransporter, as occurs in retinal cells (64).

The biphasic effects of α-lipoic acid on levels of polyol pathway metabolites deserve comment. A reasonable possibility is that this biphasic effect depends on the competition between the tricarboxylic acid cycle and the polyol pathway. In experimental diabetes, reduction of activity of pyruvate dehydrogenase complex is known to be present (65). This effect of diabetes might be at least partly induced by oxidative stress, as a direct inactivation of pyruvate dehydrogenase, and α-ketoglutarate by reactive oxygen species, and especially 4-hydroxynonenol, is known to occur (66,67). Concurrently, polyol pathway activity is increased (57). α-Lipoic acid has an effect on glucose uptake (this study), thereby increasing polyol pathway activity; it is known to increase Krebs cycle activity (68,69). A quantitative analysis of the effects of α-lipoic acid on the two pathways is not possible at this time, since dose effect information is not available in nerve on the

effects of the drug on Krebs cycle. The situation is further complicated by the opposite effects of the R- and S-enantiomers of α-lipoic acid (68). Overall, α-lipoic acid in the doses we used in this study are within the range that is known to dose-dependently correct the deficits in nerve conduction, nerve perfusion, and reduced glutathione (35), and will improve lipid peroxidation (35), suggesting that the net effect is beneficial. It is not certain from our own data whether the effects of α-lipoic acid benefit peripheral nerve from its effects on correcting the perfusion deficit, oxidative stress, improving PKC, *myo*-inositol levels, or energy metabolism. The quantitative importance of these or other mechanisms of α-lipoic acid await further studies.

Certain limitations of the study are relevant. We did not measure polyol pathway activity directly, and overactivity of the polyol pathway is surmised based on measurements of its metabolites and the known Km of the two enzymes involved. Although competition between entry into and activity of the Krebs cycle with polyol pathway activity is a reasonable hypothesis to explain the biphasic response to increasing doses of α-lipoic acid, we have not measured activity of the relevant enzymes under the relevant experimental conditions. The findings of this study of an increase in glucose uptake and polyol metabolites, together with the known ability of α-lipoic acid to increase pyruvate dehydrogenase and α-ketoglutarate activity in a number of nonneural tissues (68,70), suggest that the quantitative competing effects of α-lipoic acid on the polyol pathway and the Krebs cycle are worthy of future exploration. PKC is increased in both the cytosolic and membrane fractions, reaching significance in the cytosolic fraction ($P < 0.01$). The changes in PKC relative to sugar alcohols and α-lipoic acid are complex. The increase in PKC, to a first approximation, paralleled the increase in sugar alcohols, with perhaps some attenuation with α-lipoic acid treatment. For instance, in the D25 group, the levels of fructose and sorbitol are approximately double that of D0, but the increase in PKC over D0 is insignificant in cytosol and modest in membrane. Because we measured total PKC, changes in specific isoforms, as have been recently described (71), could easily be obscured.

The results of the diabetic state on nerve energy metabolism of sciatic nerve confirms our previous studies showing an increase in nerve lactate, presumably related to the increased dependency on anaerobic metabolism (8). α-Lipoic acid has no significant effect on normal nerve or ganglion, either in ^{14}C-deoxyglucose uptake, in polyol pathway substrates, or in energy metabolism (as seen in this study). The effects of α-lipoic acid supplementation may be beneficial to energy metabolism of diabetic nerve. Creatine phosphate is significantly reduced in the ischemic tissues of high metabolic rate, such as SCG (34); α-lipoic acid supplementation prevented this reduction. It had a variable effect on the lactate levels of diabetic nerves, increasing it in SCG and reducing it in sciatic nerve (both not statistically significant). The differences in creatine phosphate (reduction) and lactate (increase) in SCG from sciatic nerve may relate to a higher metabolic rate (five- to sevenfold) of ganglia (34). These modest changes in energy metabolites are typical of hypoxic nerve, where only modest changes are seen, even in severe hypoxia, induced by rearing rats in 10% oxygen (9).

In summary, α-lipoic acid restores the deficits in glucose uptake of diabetic peripheral nerve, increasing endoneurial glucose stores, and possibly resulting in an improvement in energy metabolism. The increase in endoneurial glucose results in biphasic dose-dependent alterations in fructose and sorbitol, but paradoxically increases rather than reduces *myo*-inositol. Many of the changes could be explainable on the basis of the effect of α-lipoic acid on improving nerve tissue blood flow (35,36).

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