The Linked Roles of Nitric Oxide, Aldose Reductase and, (Na^+,K^+)-ATPase in the Slowing of Nerve Conduction in the Streptozotocin Diabetic Rat

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Abstract

Metabolic and vascular factors have been invoked in the pathogenesis of diabetic neuropathy but their interrelationships are poorly understood. Both aldose reductase inhibitors and vasodilators improve nerve conduction velocity, blood flow, and (Na^+,K^+)-ATPase activity in the streptozotocin diabetic rat, implying a metabolic–vascular interaction. NADPH is an obligate cofactor for both aldose reductase and nitric oxide synthase such that activation of aldose reductase by hyperglycemia could limit nitric oxide synthesis by cofactor competition, producing vasoconstriction, ischemia, and slowing of nerve conduction. In accordance with this construct, *N*-nitro-L-arginine methyl ester, a competitive inhibitor of nitric oxide synthase reversed the increased nerve conduction velocity afforded by aldose reductase inhibitor treatment in the acutely diabetic rat without affecting the attendant correction of nerve sorbitol and *myo*-inositol. With prolonged administration, *N*-nitro-L-arginine methyl ester fully reproduced the nerve conduction slowing and (Na^+,K^+)-ATPase impairment characteristic of diabetes. Thus the aldose reductase-inhibitor-sensitive component of conduction slowing and the reduced (Na^+,K^+)-ATPase activity in the diabetic rat may reflect in part impaired nitric oxide activity, thus comprising a dual metabolic–ischemic pathogenesis. (*J. Clin. Invest.* 1994. 94:853–859.) Key words: diabetic neuropathy • microvascular complications • diabetic complications • peripheral neuropathy • nitric oxide synthase inhibition

Introduction

Both metabolic (1–3) and vascular (4–7) defects have been implicated in the pathogenesis of diabetic neuropathy, but the interrelationships among them are poorly understood. In the diabetic rat, metabolism of excess glucose to sorbitol by aldose reductase (AR2)[1] and then to fructose by sorbitol dehydrogenase (SDH) has been associated with reciprocal depletion of *myo*-inositol (MI) and other intracellular organic osmolytes (8), altered phosphoinositide metabolism, and reduced (Na^+,K^+)-ATPase activity in nerve; these metabolic derangements, alone or in concert, have been invoked in the aldose reductase inhibitor (ARI)-sensitive slowing of nerve conduction velocity (NCV) in acute experimental (1, 9) and (by analogy) human diabetes (2, 10). Because AR2 and SDH are NADPH:$NADP^+$ and NAD^+:NADH coupled, respectively, glucose-induced flux through these enzymes is associated with increased oxidative stress (secondary to diminished NADPH-dependent reduction of glutathione) (11) and a putative state of "pseudohypoxia" (manifested by a shift in the lactate/pyruvate ratio) (12). Endoneurial blood flow is also diminished shortly after the induction of diabetes in the rat, and vasodilator treatment or pharmacologic adrenergic sympathectomy increases endoneurial blood flow and NCV, implicating neural ischemia as well in the early and reversible slowing of NCV (5–7, 13). Moreover, ARI treatment, which restores nerve osmolyte levels (1–3, 8) and NCV (1, 2, 8, 13), also corrects decreased endoneurial blood flow (13) and impaired "endothelium-dependent" aortic relaxation (thought to be nitric oxide-[NO]-mediated) in chronically streptozotocin-diabetic (STZ-D) rats (14). Indeed several studies now suggest that microvascular disregulation in diabetes may primarily involve the "endothelial-dependent" NO-mediated component (15–18). Finally, NO synthase (NOS) inhibition reproduces the glucose-induced decrease (19) in (Na^+,K^+)-ATPase activity in isolated rabbit aortic rings in vitro (20). These observations together provide the framework for a potential novel metabolic-vascular pathogenetic matrix for diabetic neuropathy involving AR2, NO, and (Na^+,K^+)-ATPase.

The present studies were therefore undertaken to identify a potential role for AR2-related defects in NO synthesis or action in the pathogenesis of the reduced NCV and/or nerve (Na^+,K^+)-ATPase activity in the STZ-D rat model. These studies assessed the ability of a specific NOS inhibitor, *N*-nitro-L-arginine methyl ester (L-NAME), an arginine analog that competes for the en-

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1. *Abbreviations used in this paper:* AGE, advanced glycosylation end-products; AR2, aldose reductase; ARI, aldose reductase inhibitor; D, diabetic; L-NAME, *N*-nitro-L-arginine methyl ester; MI, myo-inositol; NCV, nerve conduction velocity; ND, nondiabetic; NO, nitric oxide; NOS, nitric oxide synthase; SPB, systolic blood pressure; SDH, sorbitol dehydrogenase; STZ-D, streptozotocin-diabetic.

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Table I. Comparison of Body Weight and Plasma Glucose in Rats at the Start of the Study and the Conclusion at 21 Days

	ND (n = 15)	ND:L-NAME (n = 7)	D (n = 12)	D:L-NAME (n = 16)	D:ARI (n = 11)	D:L-NAME + ARI (n = 11)
Baseline weight (g)	274±10	244±6	254±7	261±7	254±6	245±7
Day 21 weight (g)	379±10	345±7	271±14*	270±12*	269±11*	269±8*
Day 2 glucose (mmol/l)	6.6±0.4	7.1±0.2	25.6±1.6*	25.4±1.3*	25.1±1.7*	25.6±1.4*
Day 21 glucose (mmol/l)	8.2±0.4	7.4±0.4	28.9±1.3*	27.8±1.4*	24.9±1.0*	25.6±1.6*

Data given as mean±SE. * $P < 0.01$ vs control and control + L-NAME. (*ND*) Nondiabetic control; (*ND:L-NAME*) nondiabetic L-NAME–treated; (*D*) diabetic; (*D:L-NAME*) diabetic L-NAME–treated; (*D:ARI*) diabetic ARI-treated; (*D:L-NAME + ARI*) diabetic L-NAME-plus ARI-treated.

zyme's substrate binding site (21–24), and effectively inhibits both constitutive and the inducible NOS (21–24), to block the salutary effect of ARI treatment on NCV in the STZ-D rat, and to reproduce the slowed NCV of STZ-D in nondiabetic rats. L-NAME was able to prevent the beneficial effects of an ARI on NCV despite correction of nerve sorbitol and MI levels. L-NAME also slowed NCV in nondiabetic rats, though only after prolonged administration, with an attendant reduction in (Na^+,K^+)-ATPase activity. These results are consistent with the hypothesis that the ARI-sensitive component of NCV slowing in STZ-D rats is mediated in part by defects in NO, perhaps through competition between AR2 and NOS for NADPH, and may involve NO-related perturbations in endoneurial blood flow and/or nerve (Na^+,K^+)-ATPase.

Methods

Experimental design. Cesarean-delivered, barrier-sustained male albino Wistar rats weighing 200–300 g were maintained in individual air-filtered metabolic cages and given access to water and a standard synthetic diet (MI content 0.011% wt/wt) (ICN Biomedicals, Cleveland, OH) (25). Rats were acclimatized to their new environment for 1 wk before the start of the study. After an overnight fast, the rats were rendered diabetic by i.p. injection of STZ (60 mg/kg) (Upjohn, Kalamazoo, MI) in 0.2 ml of 10 mM citrate buffer, pH 5.5 (25). STZ-D was defined by a nonfasting tail-vein plasma glucose > 16.7 mM 48 h after STZ injection and at the end of the study (Beckman glucose analyzer II; Beckman Instruments, Fullerton, CA). After initial confirmation of diabetes, STZ-D and noninjected control rats were randomly distributed into the following experimental groups for the remainder of the 3-wk study: untreated nondiabetic rats (ND); nondiabetic rats given water containing 2.74 mM L-NAME (ND:L-NAME); untreated STZ-D rats (D); STZ-D rats given water containing 2.74 mM L-NAME (D:L-NAME); STZ-D rats given the ARI sorbinil (20 mg/kg by daily gavage in distilled water) (D:ARI); STZ-D rats given sorbinil plus water with 2.74 mM L-NAME (D:L-NAME + ARI); STZ-D rats given a 1% MI (wt/wt) diet and drinking water with 2.74 mM L-NAME (D:L-NAME + MI). To compare the chronic effects of NOS inhibition in nondiabetic rats with the chronic effects of STZ-D, nondiabetic rats were given 2.74 mM L-NAME for 3 mos (ND:L-NAME-chronic) and compared with untreated age-matched nondiabetic (ND-chronic) and 3 mos STZ-D (D-chronic) rats. All end-point measurements were performed by investigators unaware of treatment group assignments. Unless otherwise specified, all reagents were obtained from Sigma Chemical Co. (St. Louis, MO) and were of the highest purity available.

Measurement of NCV. Animals were lightly anesthetized by i.p. injection of 30–40 mg/kg pentobarbitol sodium. The body temperature was monitored using a rectal probe and maintained at 37°C with a warming pad. Sciatic-tibial NCV was determined noninvasively by stimulating proximally at the sciatic notch and distally at the ankle via bipolar electrodes with supramaximal stimulation (26). The proximal and distal latencies of the compound muscle action potentials, recorded via bipolar electrodes from the first interosseous muscle of the hindpaw, were measured from the stimulus artifact to the onset of the negative M-wave deflection, subtracted, and divided into the distance between the stimulating and recording electrodes giving a value for NCV in m/s.

Measurement of systolic blood pressure. Systolic blood pressure (SBP) was measured by the indirect tail-cuff technique (27) on days 0 and day 21. Rats were prewarmed with a heating pad for 3 min before 10–20 measurements were taken 30 s apart with a Programmed Electrosphygmomanometer (model PE-300; Narco BioSystems, Austin, TX) precalibrated with a mercury manometer, and recorded on a model 7D polygraph (Grass Instrument Co., Quincy, MA). SBP was defined as the mean of the last five recordings. This method was previously validated by simultaneous intra-carotid recording (27).

Measurement of sciatic nerve glucose, MI, sorbitol, and fructose. The left and right sciatic nerves were rapidly surgically exposed from the vertebral exit to the common peroneal bifurcation, excised, cleaned of adherent muscle and loose epineurial connective tissue, and immediately frozen in liquid nitrogen for subsequent determination of nerve osmolyte levels (25, 26, 28). Sciatic nerve MI, sorbitol, and fructose were determined by gas chromatography of aldonitrile acetate derivatives of lyophilized aliquots of deproteinized homogenates (2.0 ml 5% wt/vol trichloroacetic acid) of sciatic nerve containing 10 μg α-D-methyl mannopyranoside as an internal standard (25, 26, 28). A Varian 3700 gas-liquid chromatograph was equipped with a 30 m × 0.25 mm inner diameter SP-2100 fused silica capillary column with a 0.25 μm film thickness, a single flame ionization detector, a 8100 autosampler, and a Star Workstation Integrator (Varian Instruments, Sunnyvale, CA). The He carrier gas flow was 0.6 ml/min with split ratio of 15:1, and the column temperature was held for 2 min at 175°C and increased at 3°C/min to 270°C and held. Standard curves were generated daily, and recoveries routinely exceeded 95%.

(Na^+K^+)-ATPase assay. Composite and ouabain-sensitive ATPase activities were measured in crude homogenates of nerve and expressed as μmol ADP formed/gm wet wt/h by a previously described spectrophotometric enzymatic method (9). Nerve tissue was homogenized at 4°C in 2 ml of a 0.2 M sucrose–0.02 M Tris-HCl buffer, pH 7.5, with a Polytron homogenizer for at least five periods not exceeding 15 s each, then centrifuged at 100 *g* for 10 min at 4°C. The reaction was started by the addition of 20 μl of supernatant to 1.0-ml × 1.0-cm disposable cuvettes containing (final concentration) 100 mM NaCl, 10 mM KCl, 2.5 mM $MgCl_2$, 2 mM ethylene-glycol-bis(beta-aminoethyl-ether)-N,N′-tetraacetic acid, 1 mM Tris-ATP, 1 mM 3-(cyclohexylammonium)-phosphoenolpyruvate, 30 mM imidazole–HCl buffer, pH 7.3, 0.15 mM NADH, 50 μg lactate dehydrogenase, and 30 μg pyruvate kinase with or without 1.0 mM ouabain. After a 30-min stabilization period, the linear rate of oxidation of NADH was monitored at 340 nm. Ouabain-sensitive ATPase activity was previously shown to be identical to (Na^+,K^+)-stimulated ATPase activity in nerve homogenates by this method (9). Data are means±SE. Differences among groups were detected by ANOVA, and significance at the 0.05 level tested by the Student-Newman-Keuls test.

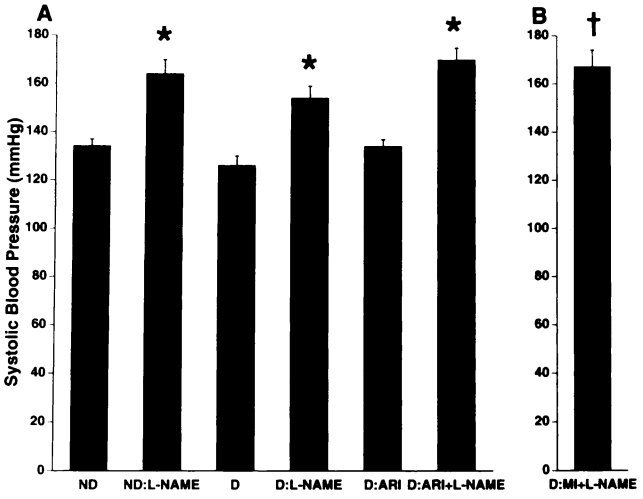

Figure 1. Change in SBP in response to 21 d of L-NAME treatment (2.74 mM/l in drinking water). SBP was measured by the indirect tail-cuff technique. The mean of five measurements was used to estimate SBP. No differences in SBP among treatment groups were observed at baseline. Data are shown as the means±SE. In *A*, * $P < 0.01$ vs. all non–L-NAME treated groups. *B* depicts an additional experiment examining the effects of a 1% MI diet plus L-NAME treatment in STZ-D rats ($^{\dagger}P < 0.01$ vs non–L-NAME treated groups).

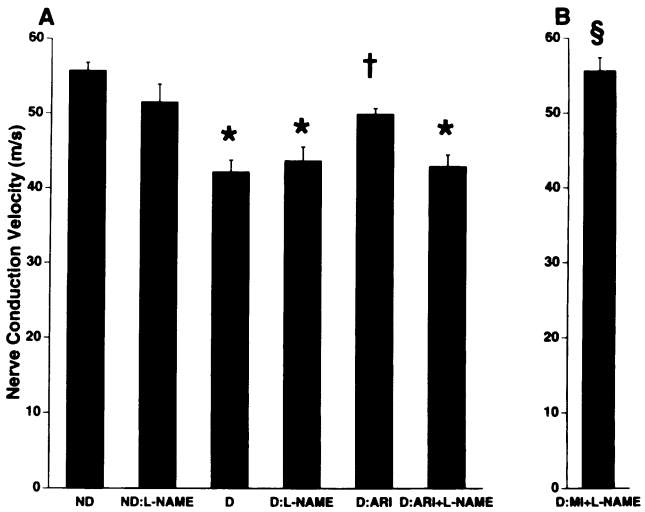

Figure 2. The effect of L-NAME on sciatic NCV in ND and STZ-D (D) rats, and its ability to block the effects of the ARI sorbinil (20 mg/kg by gavage) (*A*) or reverse the effects of a 1% MI diet (*B*). Animals were lightly anesthetized with i.p. pentobarbitol sodium and NCV was measured noninvasively in the left sciatic tibial conducting system. Data are shown as means±SE. *A*, * $P < 0.01$ vs ND, $^{\dagger}P < 0.05$ vs other STZ-D groups. *B*, $^{\dagger}P < 0.05$ vs D, P = ns vs ND.

Results

Baseline body wts were similar in all the experimental groups (Table I), but attained body wts were 21–29% lower in D vs ND groups, and unaffected by L-NAME or sorbinil. Untreated D and ND rats exhibited 14–16% increases in SBP after 21 d. As expected (21, 24, 27, 29), L-NAME increased SBP vs relevant controls from 27% in ND to 44% in D:ARI rats, with none of the SBP differences among L-NAME groups attaining statistical significance (Fig. 1).

Effect of diabetes, ARI, and L-NAME on nerve glucose, fructose, sorbitol, and MI. Nerve glucose was increased 2.8–3.4-fold in D rats, with no significant differences among D groups (Table II). Nerve fructose was increased 6.5- and 4.6-fold, respectively, in D and D:L-NAME rats vs their ND controls, and lowered by 60–62% ($P < 0.01$) by ARI (Table II). Nerve sorbitol was increased 6.5- and 6-fold, respectively, in D and D:L-NAME rats respectively and reduced by 50–69% ($P < 0.01$) by ARI (Table II). ARI corrected nerve MI in D rats to 94% of the ND level, and L-NAME had no effect on nerve osmolytes in D or ND rats (Table II).

Table II. 21 Day Sciatic Nerve Osmolyte Changes in Response to Diabetes, L-NAME and the Aldose Reductase Inhibitor Sorbinil

Group	Glucose	Fructose	Sorbitol	MI
ND	4.6±0.5	2.1±0.4	0.4±0.0	3.4±0.3
ND:L-NAME	4.6±0.7	1.6±0.3	0.3±0.0	3.4±0.3
D	15.0±2.0*	13.6±2.9*	2.6±0.4*	2.2±0.2‡
D:L-NAME	12.8±1.4*	9.7±1.6*	2.4±0.2*	2.4±0.2‡
D:ARI	13.3±1.2*	5.5±1.0§	1.3±0.2‡§	3.2±0.3‖
D:L-NAME + ARI	15.5±3.3*	5.2±0.4§	0.8±0.2§	3.2±0.3‖

Osmolyte levels given as nmol/mg protein. Data shown as mean±SEM. * $P < 0.01$; ‡ $P < 0.05$ vs control; § $P < 0.01$; ‖ $P < 0.05$ vs diabetic.

Effect of diabetes, ARI, 1% dietary MI, and L-NAME on NCV. 3 wk of L-NAME treatment produced a slight (7.5%) but statistically insignificant slowing of NCV in ND rats (Fig. 2) which was not augmented by a 10× higher L-NAME dose to correct for higher water intake in STZ-D rats (data not shown). Three wks of STZ-D slowed NCV by 24% ($P < 0.01$) (Fig. 2). L-NAME slowed NCV no further in D rats (Fig. 2). ARI increased NCV 18.8% ($P < 0.01$) in D rats to a level statistically indistinguishable from that of ND rats (Fig. 2). Despite the fact that L-NAME had no effect on NCV in untreated D rats, L-NAME decreased NCV in ARI-treated D rats to a level indistinguishable from that of untreated D rats (Fig. 2). In order to demonstrate that the effects of L-NAME on NCV in the ARI treated D animals were due to specific inhibition of NO synthesis the effects of the simultaneous addition of arginine (274 mM in drinking water) were examined in 10 animals. Arginine was found to preserve NCV at levels that were indistinguishable from the ND control animals (56.1±2.3 m/s) and prevent the hypertensive effect of L-NAME (SBP 121±5 mmHg at 3 wk). Dietary MI supplementation increased NCV in D rats given L-NAME to a level indistinguishable from that of the ND controls (55.6±1.8 m/s, $n = 5$), despite unabated hypertension (see panels *B*, Figs. 1 and 2). Thus, in contrast to its potent anti-ARI effect, L-NAME did not block the beneficial effect of dietary MI on NCV in D rats (9).

Effects of chronic (3 mos) L-NAME on NCV and nerve MI in ND rats. 3 mos of L-NAME treatment (ND:L-NAME-chronic) decreased NCV 17% ($P < 0.01$) to levels indistinguishable from rats with 3 mo of STZ-D (D-chronic) (Fig. 3). The L-NAME–induced increase in SBP after 3 mos (165±3 mmHg) was not significantly different from that after 3 wk L-NAME

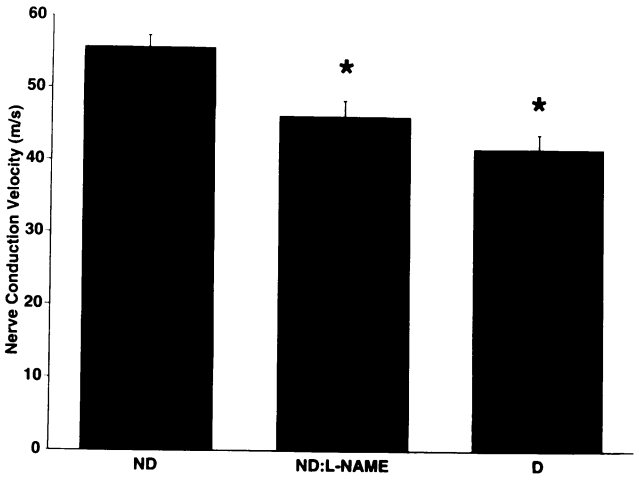

Figure 3. Effect of chronic (3 mos) L-NAME treatment on sciatic NCV in the ND rat. Data are shown as means±SE. * $P < 0.01$ vs untreated ND.

treatment. Nerve MI levels in ND:L-NAME-chronic rats were 19% lower than in untreated ND rats, but this change was statistically insignificant. To assess the specificity of the effect of ARI on reduced NCV, NCV was measured in ND rats treated for 3 mos with L-NAME + ARI ($n = 4$). ARI plus L-NAME for 3 mos did not change either SBP or NCV compared with ND animals ($n = 5$) treated with L-NAME alone (data not shown).

Effects of diabetes and L-NAME on nerve (Na^+,K^+)-ATPase activity. To assess whether NOS inhibition diminished NCV through a (Na^+,K^+)-ATPase mechanism, ouabain-sensitive ATPase activity was measured in nerve homogenates from ND-chronic, ND:L-NAME–chronic, and D-chronic rats. Ouabain-sensitive ATPase activity was reduced by 34% ($P < 0.05$) in D-chronic rats, and even more so (44%, $P < 0.05$) in the ND:L-NAME–chronic rats despite the absence of hyperglycemia in the latter (Fig. 4). In contrast, acute (3 wk) L-NAME treatment that only marginally reduced NCV in ND:L-NAME rats did not significantly reduce ouabain-sensitive ATPase activity (98.4±14.2 μmol/g/h in ND:L-NAME rats vs 106.7±14.4 μmol/g/h in ND rats, P = ns). The simultaneous treatment of ND rats with ARI plus L-NAME resulted in a similar decrease ($P < 0.05$) in (Na^+,K^+)-ATPase activity after 3 mos when compared with the ND:L-NAME-chronic rats (29 vs 22%, P = ns). Therefore changes in nerve (Na^+,K^+)-ATPase activity induced by NOS inhibition paralleled the changes in NCV in a time-dependent manner in ND rats.

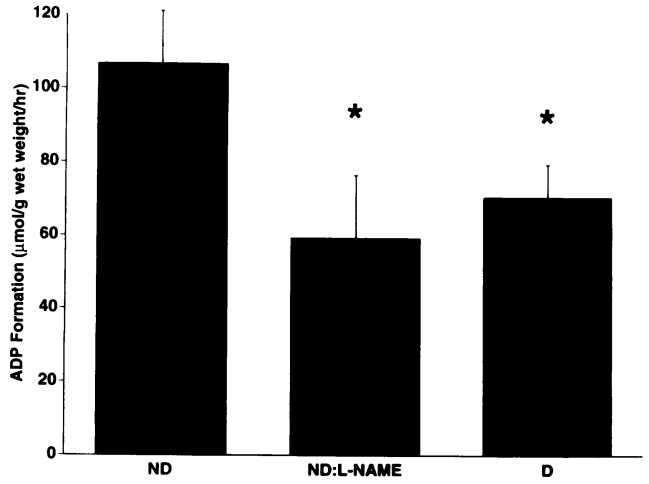

Figure 4. Changes in sciatic nerve ouabain-sensitive ATPase activity after 3 mos of L-NAME treatment in ND rats vs untreated ND controls and 3-mo STZ-D rats. Composite and ouabain-sensitive ATPase activities were measured in crude homogenates of sciatic nerve by a enzymatic–spectrophotometric techniques as described in Methods. Data are shown as means±SE. * $P < 0.05$ vs untreated ND rats.

Discussion

The seemingly divergent metabolic and vascular pathogenetic hypotheses for experimental diabetic neuropathy are becoming increasingly intertwined (1, 5). Early and rapidly reversible slowing of NCV in acutely diabetic rats, traditionally ascribed to AR2-mediated MI depletion and reduced nerve (Na^+,K^+)-ATPase activity (1, 2), may involve a prominent ischemic (4, 30) or ischemic-like (12) component. Endoneurial blood flow, reduced by 3 wk of STZ-D and remaining so for 4 mos (5), responds to treatment with ARIs, vasoactive compounds such as prostaglandin E1 analogues (13), and sympatholytic agents (6) all of which also improve NCV and/or nerve (Na^+,K^+)-ATPase activity. Moreover increased flux of glucose through AR2 and SDH may produce simultaneous oxidative stress (11) and metabolic "pseudoischemia" (12). Thus, neither temporal separation nor ARI sensitivity distinguish metabolic from vascular components of NCV slowing in experimental diabetic neuropathy.

Nitric oxide, with its increasingly appreciated functional diversity in cell metabolism, microvascular regulation, neuromodulation, and diabetes provides a strong theoretical bridge between AR2 and (Na^+,K^+)-ATPase and the vascular components of early experimental diabetic neuropathy. This study tested the hypothesis that impaired NO activity mediates the effect of increased AR2 activity on NCV and/or nerve (Na^+,K^+)-ATPase in the acutely STZ-D rat. The specific NOS inhibitor L-NAME completely blocked the salutary effects of a potent ARI on NCV in acute STZ-D rats, despite improvement in sorbitol accumulation and MI depletion; L-NAME alone had little effect on the already reduced NCV in acute STZ-D rats. These acute effects of L-NAME were prevented by arginine supplementation of the drinking water, consistent with its action as a competitive inhibitor of NOS (21–24). Chronic (12 wk) L-NAME administration to normal rats reproduced the NCV and (Na^+,K^+)-ATPase defects of chronic STZ-D, and, as anticipated, these were unaffected by ARI. Thus the ARI-sensitive slowing of NCV in acute STZ-D appears to be specific, and to some extent involve NO-related ischemic and/or metabolic (e.g., [Na^+,K^+]-ATPase) defects in nerve.

This conclusion raises three important issues: the metabolic factors linking hyperglycemia and AR2 to NOS and NO; the relative importance of vascular vs nonvascular effects of NO and/or other vasoactive modulators on NCV slowing in STZ-D; and the potential links between NO and nerve (Na^+,K^+)-ATPase. Hyperglycemia could suppress or induce local NO production or action by one of several hypothetical mechanisms. Quenching of NO by hyperglycemia-induced "advanced glycosylation endproducts" (AGE) has been described (31), and en-

hanced NO production (18) by AGE-mediated macrophage activation (32) is theoretically possible. AR2 and NOS share NADPH as an obligate cofactor; therefore enhanced glucose flux through AR2 could blunt NOS activity as a function of the relative Km's of these enzymes for NADPH and their cellular colocalization within a common glucose-accessible compartment. For example, AR2 and NOS both have been identified in vascular endothelia and autonomic ganglia (21, 33, 34); in peripheral nerve, AR2 has been localized to the Schwann cell cytoplasm (33), but the precise tissue compartmentalization of nerve NOS remains to be established. Diabetes decreases composite NADPH levels in some tissues such as lens (35), but not in whole nerve when glutathione redox state is used as a surrogate for NADPH:$NADP^+$ redox potential (36). Thus diabetes-induced shifts in NADPH:$NADP^+$ may be tissue specific and/or highly compartmentalized. Protein kinase C activation by glucose-induced de novo synthesis of diacylglycerol (37) could modulate NOS by phosphorylation–dephosphorylation (38). The increased oxidative stress of diabetes (18, 21, 39), attributed in part to increased local NADPH consumption by AR2 (11, 35) could inactivate NO through oxygen free radical formation (3), which could further impair vascular regulation in diabetes through the eicosanoid pathway (see below).

Impaired endothelium-dependent vascular relaxation has been associated with experimental (15–17) and perhaps (40) human diabetes (41), and attributed to NO depletion (15–18, 21, 39, 40, 42, 43), NO resistance (44) and/or perturbation of vasoconstricting and vasodilating eicosanoids (45, 46). The blunted endothelial-dependent vasodilatory response to acetylcholine (15, 16, 47) in the face of an intact endothelial-independent vasodilatory response to sodium nitroprusside (17), nitroglycerin (16, 18) and other NO agonists implicates defective endothelial NO synthesis or release. This would in turn alter basal vascular tone through reduced activation of guanylate cyclase (17, 21, 48, 49) or perhaps (Na^+,K^+)-ATPase in vascular smooth muscle (20).

Nitric oxide deficiency might perturb NCV and nerve (Na^+,K^+)-ATPase through a nonischemic mechanism, perhaps interacting with other more well established AR2-related defects in signal transduction (1, 47–53) involved in modulation of (Na^+,K^+)-ATPase activity (1, 2, 9, 13, 54). NO may modulate phosphoinositide turnover by unknown mechanisms (55) such that NO deficiency might act synergistically with MI depletion in altering phosphoinositide signal transduction and (Na^+,K^+)-ATPase regulation. The replication of a glucose-induced decrease in rabbit aortic (Na^+,K^+)-ATPase activity by a NOS inhibitor in vitro (20) supports this notion. Alternatively, the heightened ischemic sensitivity of neural (Na^+,K^+)-ATPase (56), and the lack of vascular autoregulation in nerve (30) might predispose peripheral nerve (Na^+,K^+)-ATPase to NO-related ischemia, possibly complicated by metabolic "pseudohypoxia" (12).

The delayed effect of L-NAME vs STZ-D on NCV, and the failure of L-NAME to block the effects of dietary MI on NCV in STZ-D rats each deserve comment. Experimental diabetes predisposes nerve fibers to ischemic insult (57), perhaps reflecting defective compensatory vasoregulatory mechanisms such as the eicosanoid pathway (46). A decrease in nerve prostacyclin in STZ-D has been speculated to favor vasoconstriction and thereby slow NCV (46, 58, 59), which would explain the salutary effects of prostaglandin E1 analogues on NCV (13) possibly via nerve cyclic AMP (60) and/or (Na^+,K^+)-ATPase (54). Glucose-induced metabolic "pseudohypoxia" might also magnify NO-related ischemia in STZ-D rats, but not in L-NAME-treated nondiabetic rats (12). Thus AR2-mediated NO depletion might initiate a complex cascade of ischemic and metabolic events that interact with other hyperglycemic/ischemic sequelae (12, 46, 61) to further compromise nerve blood flow and metabolism and degrade NCV in a self-reinforcing cycle. The delayed appearance of NCV slowing in L-NAME–treated ND rats might simply reflect the absence of these other hyperglycemic sequelae.

The failure of L-NAME to counteract the salutary effects of MI feeding on NCV in STZ-D rats implies: (*a*) that ARIs correct NCV by a mechanism at least partially independent of MI repletion (12); (*b*) that MI feeding and ARI treatment correct MI depletion in a quantitatively or qualitatively different way; or (*c*) that NO itself mediates MI repletion by ARIs. Thus ARI therapy increases endoneurial blood flow (13) which might be prevented by L-NAME; nerve blood flow has not been well studied after MI feeding. Treatment with an ARI might improve (Na^+,K^+)-ATPase function partly through a NO dependent vascular or metabolic mechanism, and partly via correction of MI and phosphoinositide metabolism (1). In contrast, MI feeding might work solely through the latter mechanism (2). Finally, ARI therapy and MI supplementation may restore NCV and/or (Na^+,K^+)-ATPase through entirely independent but parallel mechanisms. Thus the precise relationship between AR2 activation, MI depletion and NCV slowing in diabetes may be more complex than previously appreciated (1). In any case, the failure of L-NAME to slow NCV in MI-treated STZ-D rats despite persistent hypertension argues against a non-specific "neurotoxic" or hypertensive effect of L-NAME on NCV. (The lack of vascular autoregulation in nerve [30] causes nerve blood flow to fluctuate passively with blood pressure [62] such that increments in SBP would be expected to increase rather than decrease nerve blood flow.)

Although certain subtle complexities remain, these studies introduce NO as a potentially important mediator of AR2 effects on nerve (Na^+,K^+)-ATPase and NCV in experimental diabetes. Even if the direct effect of NO on nerve (Na^+,K^+)-ATPase reported in this study is not itself vascularly mediated (20), the pivotal role of NO in microvascular regulation in general still serves to conceptually unify the so-called "vascular" and "metabolic" hypotheses for experimental diabetic neuropathy.

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