

Dissection of Metabolic, Vascular, and Nerve Conduction Interrelationships in Experimental Diabetic Neuropathy by Cyclooxygenase Inhibition and Acetyl-L-Carnitine Administration

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Alterations in cyclooxygenase (COX) pathway activity have been implicated in the pathogenesis of experimental diabetic neuropathy (EDN). These studies explore the relationships between COX-mediated and acetyl-L-carnitine (ALC)-sensitive defects that contribute to functional, metabolic, and vascular abnormalities of EDN. The effects of nonselective COX inhibition with flurbiprofen were contrasted with selective COX-2 inhibition with meloxicam, administered alone and in combination with ALC in nondiabetic (ND) and streptozotocin-induced diabetic (STZ-D) rats. Flurbiprofen treatment of ND rats replicated many of the biochemical and physiological abnormalities of EDN, i.e., reduced motor nerve conduction velocity (MNCV), total and endoneurial nerve blood flow (NBF), Na,K-ATPase activity, and *myo*-inositol (MI) and taurine content. In STZ-D rats, however, flurbiprofen paradoxically prevented endoneurial NBF deficits but not MNCV slowing. Coadministration of 50 mg · kg⁻¹ · day⁻¹ ALC prevented reductions in MNCV, Na,K-ATPase activity, and endoneurial NBF in flurbiprofen-treated ND and STZ-D rats. In contrast, selective COX-2 inhibition with meloxicam was without effect on MNCV, NBF, or MI content in ND rats and prevented MNCV slowing and NBF deficits in STZ-D rats. Western blot analysis showed unchanged sciatic nerve COX-1 protein but increased COX-2 protein abundance in STZ-D versus ND rats. These results imply 1) a tonic role of the COX-1 pathway in the regulation of nerve osmolytes and Na,K-ATPase activity and the maintenance of NBF in ND animals and 2) activation of the COX-2 pathway as an important mediator of NBF and MNCV deficits in EDN. *Diabetes* 51:2619–2628, 2002

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ALC, acetyl-L-carnitine; AR, aldose reductase; COX, cyclooxygenase; EDN, experimental diabetic neuropathy; HRP, horseradish peroxidase; IC₅₀, half-maximal inhibitory concentration; MI, *myo*-inositol; MNCV, motor nerve conduction velocity; NBF, nerve blood flow; ND, nondiabetic; NF, nuclear factor; PG, prostaglandin; PKC, protein kinase C; PVDF, polyvinylidene difluoride; ROS, reactive oxygen species; RSN, rat sciatic nerve; SNH, sciatic nerve homogenates; STZ, streptozotocin; STZ-D, streptozotocin-induced diabetic; TX, thromboxane.

Diabetic peripheral neuropathy is a multifactorial disorder, attributable to the reversible metabolic consequences of hyperglycemia, insulin deficiency, or both (1) that are thought to induce further neurochemical, neurotrophic, or neurovascular defects in the peripheral nervous system. Accumulating evidence implicates increased oxidative stress (2–5) and alterations in glucose-sensitive signal transduction pathways (6) in the pathogenesis of experimental diabetic neuropathy (EDN). Hyperglycemia is proposed to promote oxidative stress and generate reactive oxygen species (ROS). In turn, increased ROS are directly neurotoxic, promoting neuronal apoptosis (7,8), and may inhibit mitochondrial respiratory enzymes, leading to deficits of nerve energy production and nerve functional deficits.

Glucose-related or “glucotoxic” pathogenetic mechanisms in EDN include nonenzymatic glycation of proteins (9), autooxidation of glucose (10), and activation of the aldose reductase (AR) pathway (6,11). Activation of the AR pathway alters cellular redox couples, exacerbates oxidative stress (6,12–14), and promotes intracellular sorbitol and fructose accumulation. Sorbitol accumulation in the diabetic nerve can lead to compensatory depletion of other nonionic organic osmolytes such as *myo*-inositol (MI) (6,15,16) and the β-amino acid taurine (17), with resultant effects on signal transduction pathways, Na,K-ATPase activity (16), and antioxidative capacity (18). Alternatively, a reduction in nerve blood flow (NBF) and the development of endoneurial hypoxia—attributed to oxidative stress (2,5,19,20) and to alterations in vasoactive agents, including endothelium-derived nitric oxide (NO) (21–23), endothelin-1 (24), and eicosanoids (23)—have been invoked as fundamental etiologic factors in EDN.

Insulin deficiency can also promote alterations in fatty acid metabolism, via blockade of the conversion of γ-linolenic acid to γ-linolenic acid (25), limiting the formation of arachidonate (25), and thereby perturbing the production of vasodilating eicosanoids including prostacyclin (26) and prostaglandin (PG) E₁ (27). In the nerve, increased oxidative stress, via induction of nuclear factor (NF)-κB (28,29) and nerve hypoxia (30), may further impair nerve perfusion by increasing cyclooxygenase (COX)-2 activity and inhibiting prostacyclin synthetase activity, leading to in-

TABLE 1
Experimental conditions and group designations (experiment 1)

Treatment	Group designation
Nondiabetic, untreated	ND
Nondiabetic, flurbiprofen-treated	ND+F
Nondiabetic, flurbiprofen/acetyl-L-carnitine-treated	ND+F+ALC
Streptozotocin-diabetic, untreated	STZ-D
Streptozotocin-diabetic, flurbiprofen-treated	STZ-D+F
Streptozotocin-diabetic, acetyl-L-carnitine-treated	STZ-D+ALC
Streptozotocin-diabetic, flurbiprofen/acetyl-L-carnitine-treated	STZ-D+F+ALC

Flurbiprofen dose, $6 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$; acetyl-L-carnitine dose, $50 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$.

creased production of vasoconstricting thromboxanes PGH_2 (31), thromboxane (TX) A_2 (27), and PGF_{2a} and reduction in vasodilating prostacyclin (32), therefore favoring vasoconstriction and ischemia. Reciprocally, COX-2 upregulation increases the rate of PGG_2 to PGH_2 conversion and ROS generation, further exacerbating oxidative stress. Finally, EDN is associated with depletion of carnitine and acetyl-L-carnitine (ALC) in peripheral nerve (33), which may further limit mitochondrial fatty acid transport and oxidation (34,35). Thus, defective fatty acid metabolism in diabetes may be both a cause and a consequence of impaired NBF, with alterations in prostanoid production and action serving as an intermediate step in both the vascular and metabolic components of diabetic peripheral neuropathy.

The studies reported herein attempt to explore the complex relationship between the COX pathway and ALC-sensitive defects underlying the characteristic functional, metabolic, and vascular abnormalities of EDN. The experimental results demonstrate the importance of COX pathway flux in the maintenance of nerve organic osmolyte content, Na,K-ATPase activity, NBF, and motor nerve conduction velocity (MNCV) and implicate a specific role for COX-2 activation in the development of neurovascular and functional deficits in EDN.

RESEARCH DESIGN AND METHODS

Animal model. Barrier-sustained, Cesarean-delivered male Wistar rats (200–300 g) were acclimatized for 1 week before being fasted overnight and rendered diabetic by an intraperitoneal (i.p.) injection of streptozotocin (STZ) (45 mg/kg) (Upjohn, Kalamazoo, MI) in 0.2 ml of 10 mmol/l citrate buffer, pH 5.5. Diabetes was defined as nonfasting plasma glucose ≥ 200 mg/dl in tail vein blood (One Touch II; Lifescan, Milpitas, CA) 48 h after STZ injection. Animals were subsequently randomly assigned to the experimental groups listed in Table 1, maintained for 4 weeks in individual air-filtered metabolic cages with ad libitum access to water, and fed a standardized rat diet (ICN Biomedicals, Cleveland, OH). After measurement of MNCV and NBF, animals were killed by exsanguination, and the sciatic nerves were rapidly excised and cleaned for biochemical measurements. Flurbiprofen, a nonselective COX-1 and -2 inhibitor (relative half-maximal inhibitory concentration [IC_{50}], COX-2 vs. COX-1: 5–10 [36,37]), was administered in the drinking water at a dose of $6 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$, with the concentration reduced in the STZ-induced diabetic (STZ-D) animals (5:1) to compensate for their increased water consumption. ALC was given by daily gavage at a dose of $50 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ (using water as vehicle), which is the lowest dose that corrects both nerve Na,K-ATPase activity and MNCV (33) and increases nerve PGE_1 levels (38). Laser-Doppler flux measurements may underestimate the endoneurial nutritive component of NBF, which may critically determine endoneurial oxygenation (3,39,40). Therefore, separate experiments (experiments 2 and 4) employed hydrogen clearance, which distinguishes nutritive from nonnutritive (shunt) NBF (40), to evaluate total versus endoneurial NBF effects of flurbiprofen, ALC, and meloxicam in nondiabetic (ND) and STZ-D rats.

Additional experiments (3 and 4) were performed to explore the potential role of diabetes-induced increased COX-2 flux on nerve organic osmolyte content, Na,K-ATPase activity, NBF, and MNCV in EDN. The selective COX-2

inhibitor meloxicam (relative IC_{50} , COX-2 vs. COX-1: 0.08 [41,42]) was given for 4 weeks ($1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ in drinking water) to ND and STZ-D rats, with the dose adjusted in STZ-D animals to ensure equality of dosing. The initial study evaluated the effects of meloxicam on nerve metabolic end points, and a second study used expanded animal groups to explore potential effects of meloxicam on nerve electrophysiology and NBF. Meloxicam was a gift from Boehringer Ingelheim Pharmaceuticals (Ingelheim/Rhein, Germany).

In all studies, investigators performing the measurements were unaware of treatment group assignments. The animal study protocols were approved by the University of Michigan Committee on Use and Care of Animals.

Measurement of sciatic nerve MI, taurine, sorbitol, and fructose content. Sciatic nerve MI, sorbitol, and fructose were determined by gas-liquid chromatography of aldonitrile acetate derivatives as previously described (17). Nerve taurine was measured by reverse-phase high-performance liquid chromatography (43). Standard curves were generated daily, and the recovery-corrected values are expressed as nanomoles per milligram wet weight of tissue.

Measurement of sciatic nerve Na,K-ATPase. Ouabain-inhibitable Na,K-ATPase activity, defined as the difference in activity before and after the addition of ouabain, was measured as previously described (17) and is expressed as micromoles ADP formed per hour per gram wet weight of tissue. **Measurement of sciatic tibial MNCV.** MNCV was measured before the determination of NBF in the contralateral limb in rats anesthetized with i.p. urethane (1–1.2 g/kg) as previously described (17). Body temperature was monitored by a rectal probe and maintained at 37°C with a warming pad. Hindlimb skin temperature was also monitored by a thermistor and maintained at 36 – 38°C by radiant heat.

Measurement of total sciatic NBF by laser-Doppler. In experiments 1 and 3, total sciatic NBF was assessed in anesthetized rats using a laser-Doppler blood flow monitor (MBF3/D; Moor Instruments, Devon, U.K.) (44). 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 large epineurial blood vessels. Care was taken not to compress 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 10–15 min before readings were taken. Body temperature was maintained at 37°C as described above, 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 at 36 – 38°C . Four flux measurements over 2 min each were obtained from the same section of nerve, and the mean value was calculated as arbitrary Doppler units. Each of the four Doppler flux measurements was the mean value of a 2-min recording period; the rats were not artificially ventilated during Doppler measurements. To take into consideration perfusion pressure variations, all flow data are expressed as vascular conductance, calculated by dividing NBF by the mean systemic blood pressure over the recording period.

Measurement of endoneurial nutritive NBF by microelectrode polarography and hydrogen clearance. In experiments 2 and 4, endoneurial nutritive NBF was assessed by hydrogen clearance as previously described (40,45) in rats anesthetized with an i.p. injection of urethane (1 g/kg). 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 two determinations at different sites was used to determine nutritive NBF.

Western blot analysis. Fresh frozen rat sciatic nerve (RSN) was lysed in radioimmunoprecipitation assay (RIPA) buffer (Tris-HCl 50 mmol/l, NaCl 150 mmol/l, EGTA 1 mmol/l, Nonidet P-40 1%, Na-deoxycholate 0.015%, aprotinin,

TABLE 2
Effects of COX inhibition, ALC, and STZ-D on body weight and blood glucose (experiment 1)

Group	n	Body weight (g)	Plasma glucose (mg/dl)
ND	10	465 ± 20	82 ± 3
ND+F	9	385 ± 12*	82 ± 3
ND+F+ALC	8	367 ± 12*	86 ± 2
STZ-D	11	332 ± 5*	356 ± 25*
STZ-D+F	14	307 ± 5*	347 ± 17*
STZ-D+ALC	13	310 ± 10*	329 ± 15*
STZ-D+F+ALC	13	298 ± 6*	343 ± 13*

Data are means ± SE. * $P < 0.05$ vs. ND.

leupeptin, pepstatin 1 $\mu\text{g/ml}$, and phenylmethylsulfonyl fluoride 1 mmol/l), and protein concentrations were measured by bicinchoninic acid protein assay kit (Sigma, St. Louis, MO). Forty-microgram aliquots of protein extract were separated by electrophoresis on a 7.5% SDS-polyacrylamide gel and transferred to a polyvinylidene difluoride (PVDF) membrane. The blots were then blocked in 5% nonfat dry milk in Tris-buffered saline for 1 h and incubated for 2 h at room temperature with goat polyclonal anti-COX-2 and anti-COX-1 antibodies (Research Diagnostic, Flanders, NJ). After washing, membranes were incubated with the appropriate horseradish peroxidase (HRP)-conjugated secondary antibody and analyzed using enhanced chemiluminescence detection kits (ECL-Plus; Amersham Pharmacia Biotech, Buckinghamshire, U.K.). The specificity of the antibody was confirmed by preincubation with the cognate peptide (which effectively competed) and an irrelevant peptide (which had no effect) (data not shown). The abundance of the sciatic nerve COX-2 and COX-1 proteins in STZ-D and ND rats was compared using a phosphorimager.

Immunohistochemistry. Goat polyclonal anti-COX-2 and anti-COX-1 antibodies were used for the immunohistochemistry studies. RSNs were cryoprotected by rinsing in 0.1 mol/l phosphate buffer, pH 7.3, containing 20% sucrose. Sections (15 μm) were cut on a cryostat, thaw-mounted onto slides coated with Superfrost (Fisher, Pittsburgh, PA), and stored at -20°C . Frozen sections were brought to room temperature and rinsed in phosphate buffer and Triton X-100 with 2% nonfat dry milk to reduce nonspecific binding of the antisera. The sections were incubated with primary antibodies (anti-COX-2 and anti-COX-1, 1:1,000 dilution) in a humid chamber for 18–24 h at 22°C . Sections were then rinsed and incubated with the appropriate biotinylated secondary antisera (goat anti-rabbit IgG, 1:500 dilution; Vector Laboratories, Burlingame, CA) followed by avidin-biotin HRP. The chromogen was developed with 3,3'-diaminobenzidine (Sigma) in the presence of H_2O_2 . The sections were counterstained with Ehrlich's hematoxylin then dehydrated through an ethanol series; cover glasses were mounted with Permount (Fisher). Immunostaining for Isolectin B4 (Vector Laboratories) was used as an anatomical marker for endothelial cells.

Statistical analysis. Data are expressed as means ± SE. Differences between experimental groups were determined by ANOVA, and the significance of between-group differences was assessed by Student-Newman-Keuls multiple range test. Significance was defined as $P \leq 0.05$. If the variances for the variables were found to differ significantly, a logarithmic transformation was performed that corrected the unequal variances. All analyses were then performed on the transformed data.

RESULTS

Effects of nonselective COX inhibition (flurbiprofen), ALC, and STZ-D on body weight and plasma glucose (experiment 1). Body weights were similar in all experimental groups at baseline. Four weeks thereafter, body weights were 29–36% lower in STZ-D rats versus ND rats ($P < 0.05$), 17% lower in flurbiprofen-treated versus untreated ND rats ($P < 0.05$), and 8% lower in flurbiprofen-treated versus untreated STZ-D rats (NS) (Table 2). Body weights were lower in both ND (21%; $P < 0.05$) and STZ-D (10%; $P = 0.4$) rats treated with flurbiprofen plus ALC versus their respective untreated controls. Body weights in STZ-D rats were not significantly de-

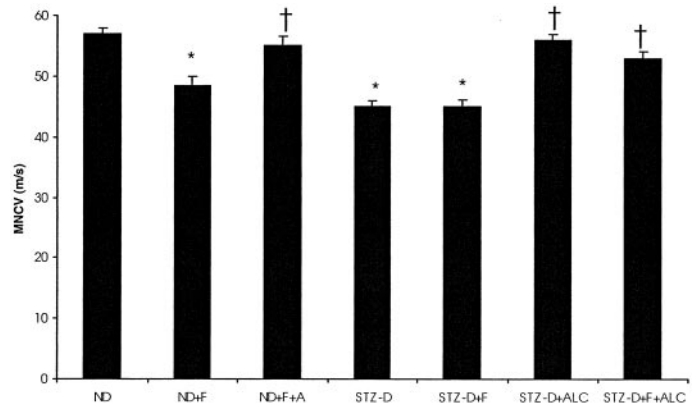


FIG. 1. Effects of nonselective COX inhibition and ALC treatment on sciatic MNCV in ND and STZ-D rats (experiment 1). During the 4-week treatment period, ND and STZ-D rats were left untreated or were treated with the nonselective COX inhibitor flurbiprofen (F) ($6 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ in drinking water) and/or ALC ($50 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ by gavage), after which MNCV was measured in the left sciatic-tibial conducting system as outlined in RESEARCH DESIGN AND METHODS. See Table 1 for explanation of experimental groups. Data are means ± SE. * $P < 0.05$ vs. ND; † $P < 0.05$ vs. STZ-D.

creased by ALC alone. Persistent hyperglycemia in STZ-D rats was unaffected by flurbiprofen or ALC (Table 2).

Effect of flurbiprofen, ALC, and STZ-D on MNCV (experiment 1). After 4 weeks, flurbiprofen decreased MNCV in ND rats by 14% versus untreated ND rats ($P < 0.05$), but this reduction was less than that produced by 4 weeks of diabetes ($P = 0.1$) (Fig. 1). As expected, MNCV was reduced by 19% ($P < 0.05$) in STZ-D versus ND rats and was not affected by flurbiprofen. The administration of $50 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ ALC corrected MNCV deficits in flurbiprofen-treated ND rats and STZ-D rats irrespective of COX inhibition.

Effects of flurbiprofen, ALC, and STZ-D on nerve polyol pathway metabolites (experiment 1). Four weeks of STZ-induced diabetes increased nerve sorbitol levels 4.9-, 4-, 2.4-, and 4.3-fold and nerve fructose levels 4.6-, 4.1-, 2.3-, and 3.1-fold in untreated, flurbiprofen-treated, ALC-treated, and flurbiprofen + ALC-treated rats versus their respective ND controls (all $P < 0.05$) (Table 3). Nerve sorbitol and fructose levels were significantly lower in the ALC-treated STZ-D group versus the other STZ-D groups. Flurbiprofen tended to lower nerve sorbitol and fructose content in ND and STZ-D rats, but this effect did not achieve statistical significance ($P = 0.2$).

Effects of flurbiprofen, ALC, and STZ-D on nerve MI and taurine content (experiment 1). Flurbiprofen lowered nerve MI and taurine content by 25 and 33%, respectively, in ND rats (both $P < 0.05$) (Table 3). Although $50 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ ALC did not affect the MI decrease, the reduction of taurine content was partially attenuated (11% higher versus flurbiprofen-treated ND; not statistically significant, $P = 0.23$). As anticipated, STZ-induced diabetes lowered nerve MI content by 47% and taurine content by 19% (both $P < 0.05$); flurbiprofen potentiated these decreases by 23% ($P < 0.05$) and 10% ($P = 0.34$), respectively. Treatment of STZ-D rats with $50 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ ALC exacerbated taurine depletion by 28% ($P < 0.05$); this potentiation was unaffected by flurbiprofen. Treatment of STZ-D rats with ALC tended to ameliorate MI depletion

TABLE 3

Effects of COX inhibition, ALC, and STZ-D on nerve organic osmolyte content and Na,K-ATPase activity (experiment 1)

Group	n	Sorbitol (nmol/mg)	Fructose (nmol/mg)	MI (nmol/mg)	Taurine (nmol/mg)	Na,K-ATPase ($\mu\text{M/g/h}$)
ND	10	0.34 \pm 0.06	1.49 \pm 0.1	4.66 \pm 0.4	4.14 \pm 0.4	155 \pm 2
ND+F	9	0.16 \pm 0.01	1.2 \pm 0.1	3.52 \pm 0.2*	2.78 \pm 0.3*	67 \pm 3*†
ND+F+ALC	8	0.32 \pm 0.04	1.41 \pm 0.15	3.69 \pm 0.1	3.11 \pm 0.2	144 \pm 3
STZ-D	11	1.67 \pm 0.17*	6.80 \pm 0.75*	2.46 \pm 0.07*	3.30 \pm 0.1*	105 \pm 7*
STZ-D+F	14	1.35 \pm 0.15*	6.08 \pm 0.52*	1.89 \pm 0.1*†	2.98 \pm 0.3*	62 \pm 5*†
STZ-D+ALC	13	0.82 \pm 0.12*†‡	3.40 \pm 0.59*†‡	2.87 \pm 0.1*	2.40 \pm 0.1*†	127 \pm 5*†
STZ-D+ALC+F	13	1.39 \pm 0.14*	4.62 \pm 0.52*‡	2.52 \pm 0.1*‡	2.28 \pm 0.3*†	123 \pm 3*†

Data are means \pm SE. Sciatic nerve sorbitol, fructose, and MI levels were determined by gas-liquid chromatography and sciatic nerve taurine by high-performance liquid chromatography as described in RESEARCH DESIGN AND METHODS. Na,K-ATPase activity was measured in sciatic nerve homogenates in the presence and absence of ouabain as described in RESEARCH DESIGN AND METHODS. The ouabain-sensitive component was taken as a measure of Na,K-ATPase activity. * $P < 0.05$ vs. ND; † $P < 0.05$ vs. STZ-D; ‡ $P < 0.05$ vs. STZ-D+F.

($P = 0.05$ vs. untreated STZ-D rats) and prevented further lowering of MI by flurbiprofen.

Effect of flurbiprofen, ALC, and STZ-D on nerve Na,K-ATPase activity (experiment 1). In ND rats, flurbiprofen profoundly decreased nerve Na,K-ATPase activity by 58% versus nontreated ND rats to levels lower than that in STZ-D rats (both $P < 0.05$); this decrease was completely corrected by ALC (Table 3). Nerve Na,K-ATPase activity was decreased by 32% ($P < 0.01$) in STZ-D rats and further decreased by flurbiprofen ($P < 0.05$). Administration of 50 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ ALC significantly increased Na,K-ATPase activity in STZ-rats, and this effect was not blocked by flurbiprofen.

Effect of flurbiprofen, ALC, and STZ-D on total NBF, assessed using laser-Doppler (experiment 1). In ND rats, flurbiprofen decreased total NBF and vascular conductance by 29 and 33%, respectively (both $P < 0.05$) (Table 4). In contrast to the flurbiprofen-induced reductions in MNCV and Na,K-ATPase activity in ND rats, the reduction of vascular conductance by flurbiprofen was not corrected by 50 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ ALC. In STZ-D rats, total NBF was reduced by 36% ($P < 0.05$ vs. ND) and further decreased by flurbiprofen. The deficit of total NBF in STZ-D rats was not affected by 50 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ ALC. When corrected for systemic blood pressure (which was 3% lower in STZ-D rats), total vascular conductance was reduced by 44% in STZ-D rats ($P < 0.05$ vs. ND). Total vascular conductance in STZ-D rats was unaffected by 50 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ ALC or flurbiprofen, alone or in combination.

Effect of ALC, flurbiprofen, and STZ-D on endoneurial nutritive and total NBF, assessed using microelectrode polarography and hydrogen clear-

ance (experiment 2). As in experiment 1, 4 weeks of STZ-diabetes interfered with weight gain and produced persistent elevation of plasma glucose that was unaffected by either ALC or flurbiprofen (data not shown). The 25% reduction in MNCV in STZ-D rats was again normalized by 50 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ ALC irrespective of flurbiprofen treatment.

In flurbiprofen-treated ND rats, total NBF assessed by hydrogen clearance was reduced by 27% ($P < 0.05$ vs. untreated ND) (data not shown). These data are consistent with the magnitude of the changes obtained by laser-Doppler flowmetry. Total NBF in STZ-D rats was reduced by 42% ($P < 0.05$ vs. ND). The deficit of total NBF in STZ-D rats with or without flurbiprofen treatment was not corrected by 50 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ ALC. These results were not affected after correction for blood pressure (which was 8–15% lower in untreated and treated STZ-D rats; $P < 0.05$ vs. ND) (expressed as vascular conductance).

In flurbiprofen-treated ND rats and untreated STZ-D rats, the endoneurial nutritive component of the NBF (Table 5) was found to decrease in parallel to total NBF. But in contrast to the measurements of total NBF, the flurbiprofen-induced reduction of endoneurial NBF in ND rats was selectively corrected by ALC (Table 5). These results were unchanged when expressed as vascular conductance. In contrast to its effects in ND rats, flurbiprofen treatment of STZ-D rats paradoxically prevented endoneurial NBF deficits. Thus, nonspecific COX inhibition in ND and untreated STZ-D rats decreased both total NBF and endoneurial nutritive NBF in parallel. However, the effects of ALC (in flurbiprofen-treated ND and STZ-D rats) and flurbiprofen (in STZ-D rats) were restricted to the

TABLE 4

Effects of nonselective COX inhibition, ALC, and STZ-D on total NBF (laser Doppler) (experiment 1)

Group	n	Total NBF (flow units)	Mean systemic BP (mmHg)	VC (flow units/mmHg)
ND	10	197 \pm 12	118 \pm 5	1.8 \pm 0.05
ND+F	9	139 \pm 8*	116 \pm 5	1.2 \pm 0.07*
ND+F+ALC	8	164 \pm 7*†	125 \pm 16	1.3 \pm 0.08*
STZ-D	11	127 \pm 7*	114 \pm 3	1.0 \pm 0.04*
STZ-D+F	13	122 \pm 8*	98 \pm 6	1.0 \pm 0.02*
STZ-D+ALC	13	134 \pm 8*	109 \pm 7	1.1 \pm 0.03*
STZ-D+F+ALC	13	126 \pm 5*	120 \pm 3	1.0 \pm 0.03*

Data are means \pm SE. Total NBF was assessed by laser-Doppler technique as described in RESEARCH DESIGN AND METHODS. All flow data were also expressed as vascular conductance (VC), which was calculated by dividing the blood flow by the mean systemic blood pressure (BP) over the recording period. * $P < 0.05$ vs. ND; † $P < 0.05$ vs. ND+F.

TABLE 5

Effects of nonselective COX inhibition, ALC, and STZ-D on endoneurial-nutritive NBF (hydrogen clearance) (experiment 2)

Group	n	Endoneurial NBF (flow units)	BP (mmHg)	Endoneurial VC (flow units/mmHg)
ND	14	14 ± 1	117 ± 3	0.12 ± 0.007
ND+F	5	8 ± 2*	132 ± 5	0.05 ± 0.02*
ND+F+ALC	8	13 ± 1†	109 ± 9	0.12 ± 0.01
STZ-D	12	6 ± 1*	121 ± 3	0.05 ± 0.005*
STZ-D+F	8	11 ± 1†	101 ± 5‡	0.11 ± 0.009†
STZ-D+ALC	6	9 ± 1†	115 ± 7	0.08 ± 0.01†
STZ-D+ALC+F	6	10 ± 1†	131 ± 5	0.08 ± 0.01†

Data are means ± SE. Endoneurial NBF was assessed by hydrogen clearance technique as described in RESEARCH DESIGN AND METHODS. All flow data were also expressed as vascular conductance (VC), which was calculated by dividing the blood flow by the mean systemic blood pressure over the recording period. * $P < 0.05$ vs. ND and ND+F+ALC; † $P < 0.05$ vs. STZ-D and ND+F; ‡ $P < 0.05$ vs. ND+F and STZ-D+ALC+F.

endoneurial nutritive component, with total NBF remaining unchanged.

Effect of selective COX-2 inhibition (meloxicam) and STZ-D on body weight and plasma glucose (experiment 3). Body weights were similar in all experimental groups at baseline (although ~100 g higher in the first subset of animals compared with experiments 1 and 2). After 4 weeks of STZ-induced diabetes, body weights were 27% lower versus ND rats in the first subset of animals and 15% lower in the second subset (data not shown). Meloxicam did not affect body weight in ND or STZ-D rats. Plasma glucose was increased by 3.4-fold in untreated STZ-D (ND 127 ± 15 vs. STZ-D 434 ± 23 mg/dl; $P < 0.05$) and was unaffected by meloxicam in ND rats (99 ± 12 mg/dl) or STZ-D rats (375 ± 38 mg/dl; $P < 0.05$ vs. ND rats with and without meloxicam treatment).

Effect of meloxicam and STZ-diabetes on nerve polyol pathway metabolites and nerve osmolytes (experiment 3). In ND rats, meloxicam treatment did not significantly effect sciatic nerve MI and fructose content but induced a 25% reduction in taurine content ($P = 0.06$) and a 60% reduction in sorbitol content ($P < 0.05$) (Table 6). Four weeks of STZ-induced diabetes increased nerve sorbitol and fructose levels twofold (both $P < 0.05$) and lowered nerve MI content by 29% and nerve taurine content by 35% (both $P < 0.05$) versus ND rats. Neither the alterations of nerve polyol pathway metabolites nor nerve osmolytes in STZ-D rats were affected by meloxicam treatment.

Effect of meloxicam and STZ-diabetes on nerve Na,K-ATPase activity (experiment 3). In ND rats, meloxicam reduced Na,K-ATPase activity by 33% ($P < 0.05$), but this reduction was significantly less than that induced by the nonselective COX inhibitor, flurbiprofen ($P < 0.05$) (Table

6). In STZ-D rats, Na,K-ATPase activity was decreased by 35% ($P < 0.05$) and was not significantly effected by meloxicam. Therefore, in contrast to flurbiprofen, selective COX-2 inhibition with meloxicam did not worsen metabolic deficits in STZ-D rats.

Effect of meloxicam and STZ-D on MNCV (experiment 3). As observed in experiment 1, 4 weeks of STZ-diabetes significantly slowed MNCV in untreated rats by 16% ($P < 0.05$) (Fig. 2). Meloxicam did not reproduce the flurbiprofen-induced slowing of MNCV in ND rats and prevented MNCV deficits in STZ-D rats.

Effect of selective COX inhibition and STZ-diabetes on total (laser-Doppler) and endoneurial (hydrogen clearance) NBF (experiments 3 and 4). In contrast to the effects of flurbiprofen, selective COX-2 inhibition with meloxicam did not decrease total NBF in ND rats (meloxicam-treated ND, 163 ± 13; untreated ND, 164 ± 10 flux units) (Fig. 3). Consistent with the findings of experiment 1, total NBF was decreased by 42% ($P < 0.01$) after 4 weeks of diabetes (94 ± 11 flux units). In STZ-D rats, in contrast to the effects of flurbiprofen, the selective COX-2 inhibitor meloxicam prevented deficits of total NBF (139 ± 13 flux units; $P < 0.05$ vs. untreated STZ-D rats). Mean systemic blood pressure was significantly lower in meloxicam-treated ND versus untreated ND rats (102 ± 4 vs. 116 ± 5 mmHg; $P < 0.05$) and in both STZ-D rat groups ($P < 0.05$ vs. ND), which did not differ significantly from each other (untreated STZ-D, 100 ± 5; meloxicam-treated STZ-D, 105 ± 5 mmHg). Therefore, when total NBF was corrected for differences in mean systemic blood pressure and expressed as vascular conductance, flow was decreased by 36% ($P < 0.01$) in untreated STZ-D rats, a deficit that was prevented by meloxicam (Fig. 3A).

TABLE 6

Effects of selective COX inhibition and STZ-D on nerve organic osmolyte content and Na,K-ATPase activity (experiment 3, subset 1)

Group	n	Sorbitol	Fructose	MI	Taurine	Na,K-ATPase
ND	11	0.05 ± 0.01	0.03 ± 0.01	0.24 ± 0.04	2.31 ± 0.3	133 ± 12
ND+M	7	0.02 ± 0.01*	0.02 ± 0.01	0.21 ± 0.04	1.74 ± 0.2	89 ± 20*
STZ-D	9	0.1 ± 0.01*	0.06 ± 0.01*	0.17 ± 0.03*	1.5 ± 0.2*	86 ± 6*
STZ-D+M	7	0.08 ± 0.01*	0.06 ± 0.04*	0.15 ± 0.01*	1.64 ± 0.1*	70 ± 9*

Data are means ± SE. Sciatic nerve sorbitol, fructose, and MI were determined by gas-liquid chromatography and sciatic nerve taurine by high-performance liquid chromatography as described in RESEARCH DESIGN AND METHODS. Na,K-ATPase activity was measured in sciatic nerve homogenates in the presence and absence of ouabain as described in RESEARCH DESIGN AND METHODS. The ouabain-sensitive component was taken as a measure of Na,K-ATPase activity. * $P < 0.05$ vs. ND.

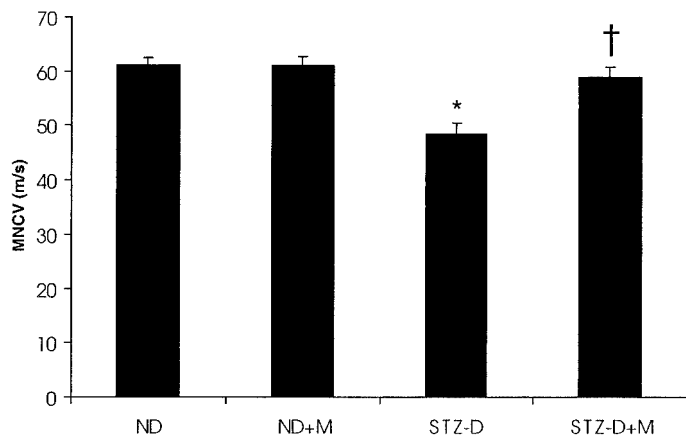


FIG. 2. Effect of selective COX inhibition and STZ-D on MNCV (experiment 3). During the 4-week treatment period, ND and STZ-D rats were left untreated or were treated with the selective COX-2 inhibitor meloxicam ($1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ in drinking water), after which MNCV was measured in the left sciatic-tibial conducting system as outlined in RESEARCH DESIGN AND METHODS. Data are means \pm SE; $n = 7$ –11 animals in each group. ND+M, ND treated with meloxicam; STZ-D+M, STZ-D treated with meloxicam. * $P < 0.05$ vs. ND; † $P < 0.05$ vs. STZ-D.

A separate experiment (experiment 4) determined the effects of meloxicam on endoneurial NBF in ND and STZ-D rats. As before, body weights of STZ-D rats were decreased by $\sim 27\%$ compared with ND animals, and meloxicam did not affect body weight or plasma glucose in ND or STZ-D rats (data not shown). Meloxicam did not decrease endoneurial blood flow in ND rats (meloxicam-treated ND, 15 ± 0.7 ; untreated ND, 15 ± 0.6 flow units). Endoneurial blood flow was decreased by 47% ($P < 0.01$) after 4 weeks of diabetes (7.9 ± 0.4 flow units). Consistent with the results of experiment 3, meloxicam prevented deficits of endoneurial NBF in STZ-D rats (15 ± 1 flow units; $P < 0.01$ vs. untreated STZ-D rats). Mean systemic blood pressure was higher in meloxicam-treated ND rats versus untreated ND rats (144 ± 3 vs. 117 ± 5 mmHg; $P < 0.05$), unaffected in untreated STZ-D rats (112 ± 8 mmHg), and lower in meloxicam-treated STZ-D rats (89 ± 1 mmHg; $P < 0.05$ vs. other groups). Therefore, when endoneurial NBF was corrected for differences in mean systemic blood pressure and expressed as vascular conductance, flow was decreased by 44% ($P < 0.01$) in untreated STZ-D rats, a deficit that was prevented by meloxicam (Fig. 3B).

Effect of STZ-D on sciatic nerve COX-1 and COX-2 protein abundance. Western blot analysis of composite sciatic nerve homogenates (SNH) showed that the polyclonal anti-COX-2 antibody recognized a single appropriately sized band of ~ 75 kDa, which is the expected molecular weight of COX-2 protein (Fig. 4). In ND rats, low-level ($n = 3$) or undetectable ($n = 3$) COX-2 expression was found in the SNH, which contrasted markedly with increased expression (94% increase versus ND rats) observed in SNH from all STZ-D rats (Fig. 4A).

In contrast, COX-1 was detectable in SNH from all rats, and there was no difference in COX-1 protein expression between STZ-D and ND rats (Fig. 4B).

Immunohistochemical localization of sciatic nerve COX. By immunohistochemical analysis of STZ-D rat sciatic nerve, COX-2 was found to be mainly localized within the vascular endothelial cells of the peri- and endoneurium (Fig. 5A). Endothelial cell localization was

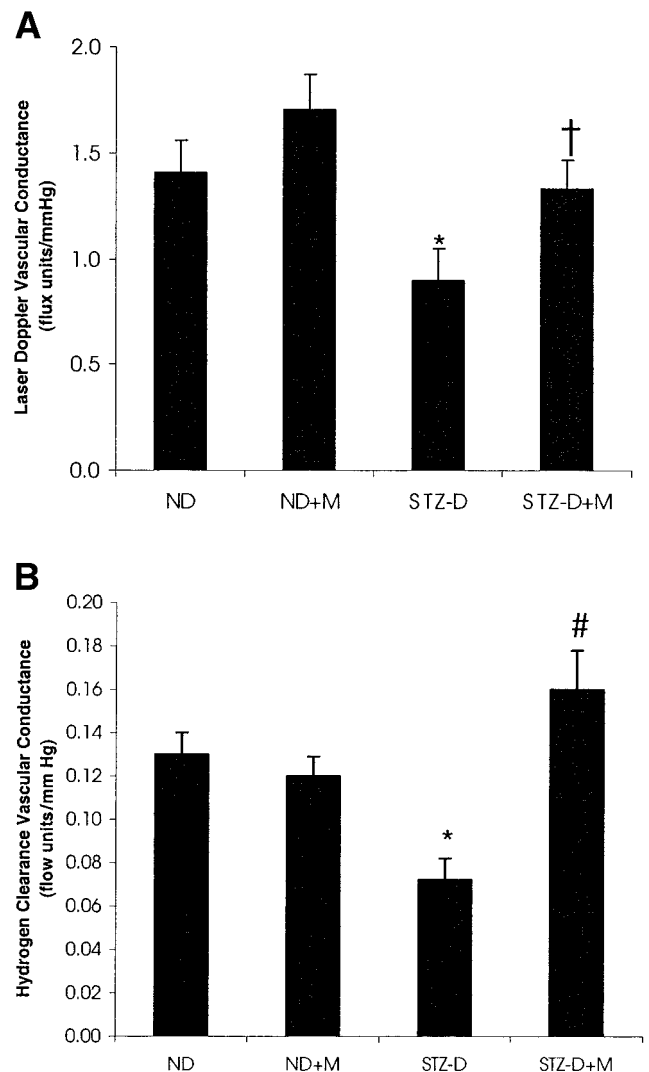


FIG. 3. Effect of selective COX-2 inhibition and STZ-D on total (laser-Doppler) (A) and endoneurial (hydrogen clearance) (B) NBF (expressed as vascular conductance) (experiment 3). During the 4-week treatment period, ND and STZ-D rats were left untreated or were treated with the selective COX-2 inhibitor meloxicam ($1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ in drinking water), after which NBF (expressed as vascular conductance [flux or flow units/mean systemic blood pressure]) was measured by laser-Doppler flowmetry or hydrogen clearance as outlined in RESEARCH DESIGN AND METHODS. ND+M, ND treated with meloxicam; STZ-D+M, STZ-D treated with meloxicam. Data are means \pm SE; $n = 6$ –14 animals in each group. * $P < 0.05$ vs. ND; † $P < 0.05$ vs. STZ-D; # $P < 0.05$ vs. other groups.

confirmed by labeling serial adjacent sections with an antibody to Isolectin B4 as described in RESEARCH DESIGN AND METHODS (Fig. 5B). In contrast, COX-1 was found to be diffusely distributed across nerve tissue compartments, without specific localization at any site (data not shown).

DISCUSSION

Alterations in COX pathway activity, potentially secondary to systemic or local defects in fatty acid metabolism in diabetes, have been implicated in endoneurial hypoxia and impaired nerve function in EDN (38,46). However, the role of the COX pathway in the regulation of nerve metabolism and function has not been fully characterized. In the studies reported herein, we explore the relationships between the COX-mediated and ALC-sensitive defects that

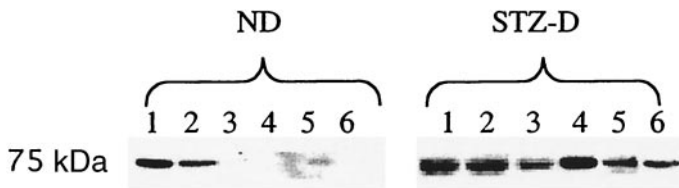
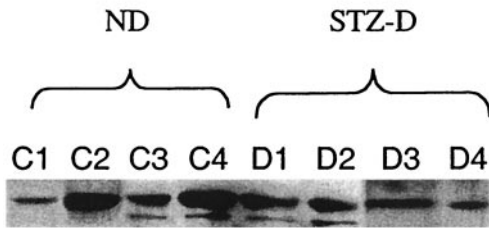
A COX-2**B COX-1**

FIG. 4. Western blot analysis of COX protein abundance in ND and STZ-D rat sciatic nerve. Protein extracts of rat sciatic nerve were separated by electrophoresis on a 7.5% SDS-polyacrylamide gel and transferred to a PVDF membrane. After blocking, blots were incubated with polyclonal anti-COX-2 (**A**) and anti-COX-1 (**B**) antibodies, washed, and incubated with the appropriate HRP-conjugated secondary antibody (see RESEARCH DESIGN AND METHODS for details). Blots were analyzed with a luminol chemiluminescence detection kit.

contribute to functional, metabolic, and vascular abnormalities of EDN.

Nonselective inhibition of the COX pathway with flurbiprofen in ND rats replicated, and in STZ-D rats potentiated, many (but not all) of the biochemical and physiological abnormalities of EDN, i.e., reduced MNCV, Na,K-ATPase activity, and MI and taurine content

(3,16,17,40,46,47). In ND rats, flurbiprofen reproduced the effects of STZ-diabetes on both total and endoneurial NBF. In STZ-D rats, however, flurbiprofen selectively prevented the deficit of endoneurial NBF (but not MNCV slowing). Co-administration of ALC with flurbiprofen prevented reductions of MNCV and Na,K-ATPase activity, implying that the flurbiprofen-induced deficits were not due to nonspecific drug toxicity. In contrast, selective COX-2 inhibition with meloxicam was without affect on MNCV, NBF, or MI content in ND rats and prevented MNCV slowing and total and endoneurial NBF deficits in STZ-D rats. Western blot analysis showed unchanged sciatic nerve COX-1 protein but increased COX-2 protein abundance in STZ-D rats compared with ND rats. These results imply 1) a tonic role of the COX-1 pathway in the regulation of nerve osmolytes and Na,K-ATPase activity and the maintenance of NBF in ND animals and 2) activation of the COX-2 pathway as an important mediator of NBF and MNCV deficits in EDN.

In animal models of EDN, the effects of COX inhibition on neurovascular and functional deficits have been inconsistent. For example, nonselective COX inhibition with indomethacin has been reported to correct slowed sural sensory NCV and increase caudal motor NCV and NBF (but not endoneurial oxygenation) to levels in excess of ND animals, despite the failure to demonstrate motor NCV slowing or NBF deficits in untreated diabetic rats (48). Additionally, the nonselective COX inhibitor sulindac corrected conduction deficits in sensory caudal and sural fibers in diabetic rats and prevented the reduction of dorsal root ganglia blood flow and sciatic nerve endoneurial oxygen tension but did not affect modest caudal MNCV slowing (49). Piroxicam has been reported to attenuate the diabetes-induced decline of sensory nerve action potential

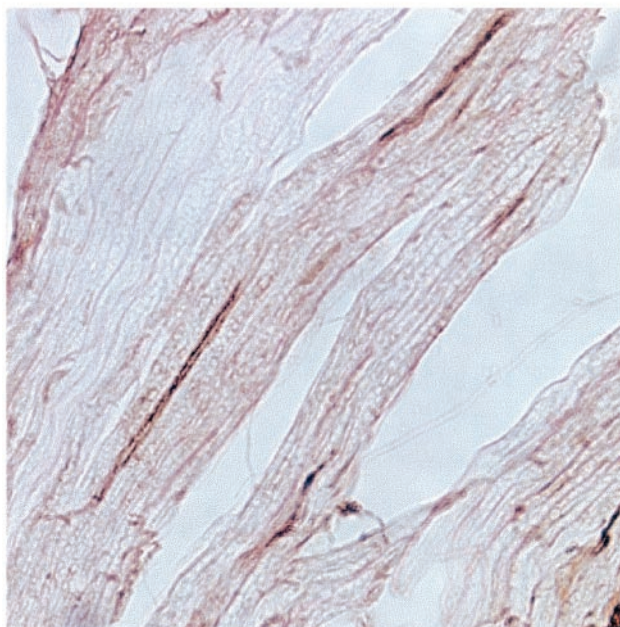
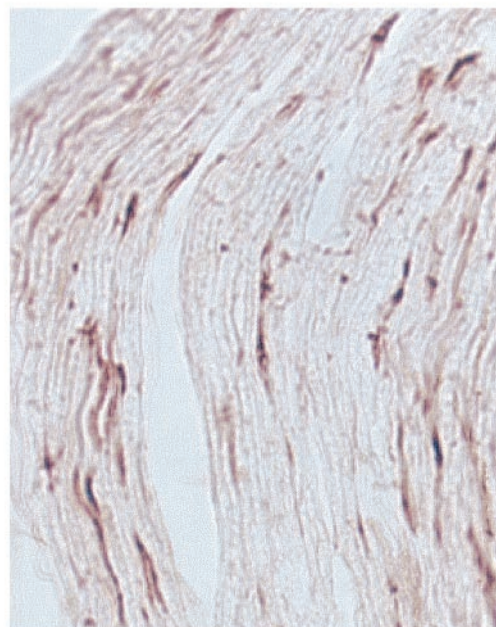
A COX-2**B Isolectin B4**

FIG. 5. Immunohistochemical localization of COX-2 in rat sciatic nerve. Goat polyclonal antibody against COX-2 (**A**) was used to immunostain fresh frozen sections of STZ-D rat sciatic nerve (see RESEARCH DESIGN AND METHODS). Immunostaining for Isolectin B4 (**B**) was used as an anatomical marker for endothelial cells.

amplitude after 16 weeks of STZ-induced diabetes but was without effect on either motor or sensory NCV (50). In this article, the lack of any beneficial effect of flurbiprofen on MNCV despite preventing endoneurial perfusion deficits in diabetic rodents is consistent with the above reports and suggests that detrimental (but ALC-sensitive) nonvascular effects of nonselective COX inhibition on nerve electrophysiology may negate improvements in endoneurial perfusion. Moreover, the effects of flurbiprofen in ND rats are consistent with rare findings reported in nondiabetic humans, in whom treatment with nonselective COX inhibitors, at doses similar to the ones used herein, has been associated with the development of peripheral somatic neuropathy (51–53). The reasons for the relatively greater susceptibility of rodents compared with humans are, however, unknown.

The explanation for the apparent inconsistency of the effects of different COX inhibitors on NCV deficits in STZ-D rats is unknown but may reflect differences in the relative degree of inhibition of the two COX pathways (or other unspecified metabolic effects). For example, flurbiprofen is a very potent COX-1 inhibitor, its potency being higher than that of indomethacin (54–56), and so any beneficial effects of the other less effective COX-1 inhibitors in diabetic animals may be attributable to the relative degree of COX-1 versus COX-2 inhibition at the dose used. This explanation would be consistent with the beneficial effects of meloxicam in EDN and would implicate an important role for the COX-1 pathway in the maintenance of nerve metabolism, NBF, and ultimately nerve conduction. Alternatively, the beneficial effects of sulindac on MNCV slowing have been attributed to inhibition of the polyol pathway (49). In this report, meloxicam did not affect nerve sorbitol levels in diabetic rats, thereby making unlikely a mechanism attributable to aldose reductase inhibition.

In ND rats, combined COX-1 and COX-2 inhibition with flurbiprofen decreased both total and endoneurial NBF, whereas selective COX-2 inhibition was without measurable effect, suggesting that tonic COX-1, but not COX-2, activity is required for the maintenance of normal NBF. In STZ-D rats, however, flurbiprofen and meloxicam both prevented endoneurial NBF deficits. This is consistent with the construct that diabetes-induced COX-2 activation may predominantly contribute to nerve perfusion deficits. This view is supported by nerve Western blot analysis demonstrating increased abundance of only the COX-2 protein in STZ-D rats and its immunohistochemical localization to the nerve vasculature. COX-1 is constitutively expressed in all cells and tissues, including peripheral nerve (57), whereas COX-2 is inducible by proinflammatory stimuli (58), oxidative stress (59), and hypoxia (30,60). However, the COX-ROS interplay is complex, since activation of COX-2 (61) can itself increase ROS production during PGG₂ to PGH₂ conversion, which generates hydrogen peroxide (58,62), therefore completing a vicious circle. Thus, in EDN, increased oxidative stress (2,5,9,63,64) would be expected to induce COX-2 expression and further exacerbate oxidative stress. In addition, induction of COX-2 in the extrinsic epineurial or perineurial penetrating vasculature may increase synthesis of vasomodulatory agents such as TXA₂, thereby facilitating

vasoconstriction, platelet aggregation, endoneurial hypoxia, and nerve conduction deficits.

Correction of endoneurial blood flow deficits has been viewed as a prerequisite for the correction of MNCV slowing in diabetic rodents (19,40,64). Measurements of NBF are not standardized and are typically performed by either laser-Doppler flowmetry or hydrogen clearance. In this article, the two techniques were consistent with each other in detecting changes of total NBF. However, laser-Doppler flux measurements may underestimate the endoneurial nutritive component of NBF, which may critically determine endoneurial oxygenation (3,40,65). Therefore, hydrogen clearance, which distinguishes nutritive from nonnutritive (shunt) NBF (66), was used to evaluate potential dissociative effects of ALC and flurbiprofen on total and endoneurial NBF in ND and STZ-D rats. In STZ-D rats, ALC and flurbiprofen selectively improved the endoneurial component of NBF, whereas total NBF remained unchanged. However, the salutary effect of ALC but not flurbiprofen in preventing MNCV slowing in STZ-D rats demonstrates the importance of the additional nonvascular, but ALC-sensitive, actions of flurbiprofen on nerve electrophysiology.

Flurbiprofen dissociated nerve perfusion and nerve conduction deficits in STZ-D rats. In 6-week STZ-D rat sciatic nerve, decreased GSH (reduced glutathione), mitochondrial and cytoplasmic NAD⁺/NADH ratios (64,67), and vasodilator-sensitive reductions of PCr/Cr and ATP/ADP ratios (67) are consistent with impaired mitochondrial oxidative phosphorylation, secondary to oxidative stress and ischemic hypoxia. Reduced mitochondrial fatty acid oxidative capacity may exacerbate accumulation of long-chain fatty acids and fatty acid esters (34,35), which are thought to perturb membrane stability and function (34), signal transduction pathways (68), and Na,K-ATPase activity (16). Administration of flurbiprofen to animals could directly reproduce (or exacerbate) the inhibitory effects of diabetes on mitochondrial fatty acid oxidation independently of endoneurial ischemia. In rats, flurbiprofen at doses similar to those used herein induces a potent inhibition of mitochondrial fatty acid oxidation via a nonstereoselective non-CoA mechanism (69,70). However, it is unknown whether the rat nerve accumulates sufficient concentrations of flurbiprofen to exert this effect *in vivo*. Moreover, the sensitivity of flurbiprofen-induced deficits in nerve metabolic and electrophysiologic function to ALC, which can enhance mitochondrial fatty acid oxidation (33–35,71), supports these hypothetical constructs. Future studies are needed to explore changes in nerve redox potentials in response to nonselective COX inhibition.

Two findings, the unanticipated and profound depletion of nerve MI and taurine content by flurbiprofen and the selective depletion of taurine by meloxicam in ND rats, implicate COX pathway activity in the regulation of nerve organic osmolyte content. The Na⁺-dependent uptake mechanisms for MI and taurine (72,73) and their efflux via volume-sensitive organic osmolyte channels are critically regulated by signal transduction pathways. Depletion of intracellular MI may impair activation of protein kinase C (PKC) in some diabetic tissues, including the neuron (6), whereas depletion of taurine in diabetes (17,18,45) may

contribute to oxidative stress (18,45), intracellular calcium overload, and activation of PKC (74). Regulation of nerve organic osmolytes is more complex than originally proposed, since levels of both MI and taurine in the nerve have been shown to be altered by ALC (33), oxidative stress (64), and now COX-pathway activity, in addition to the osmotic consequences of sorbitol accumulation (17).

Nonselective COX inhibition depressed nerve Na,K-ATPase activity in ND rats to levels below that of 4-week STZ-diabetes and further exacerbated the reduction in diabetic rats. Our finding, a reduction in nerve Na,K-ATPase activity induced by selective COX-2 inhibition in both ND and STZ-D rats, confirms the importance of COX-2 products in the maintenance of Na,K-ATPase activity. In addition to regulation through a cAMP-dependent protein kinase pathway (75,76), decreased Na,K-ATPase activity may reflect a COX inhibitor-exacerbated nerve energy deficit (77), secondary to disruption of mitochondrial function. The ability of ALC to ameliorate deficits in Na,K-ATPase activity despite potent COX inhibition is again consistent with a direct mitochondrial action (33–35,71) or possibly an effect mediated via COX-independent production of PGE₁ (78) or reduction in oxidative stress (79,80).

In summary, these studies imply 1) an important role for the COX-1 pathway in the tonic regulation of nerve osmolytes, Na,K-ATPase activity, and nerve perfusion in healthy animals and 2) pathological activation of the COX-2 pathway as an important mediator of NBF and MNCV deficits in EDN. Future studies examining the role of selective COX-2 inhibition in the management or prevention of diabetic peripheral somatic neuropathy appear warranted.

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