The Influence of Sulindac on Experimental Streptozotocin-Induced Diabetic Neuropathy

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Abstract: We studied the influence of sulindac, a nonsteroidal anti-inflammatory agent on experimental streptozotocin-induced diabetic neuropathy. Untreated diabetic rats were compared with nondiabetic rats, diabetic rats treated with low dose insulin and diabetic rats given sulindac (6.0 mg/kg by gavage 5 of 7 days weekly). Neuropathy was assessed by following serial *in vivo* motor and sensory caudal conduction, resistance to ischemic conduction failure, and *in vitro* conduction in sural myelinated and unmyelinated sensory fibers. The impact of low dose insulin and sulindac treatment on the microenvironment of the L4 dorsal root ganglion and sciatic endoneurium was asssessed by measuring local perfusion and oxygen tension after 16 weeks of diabetes. Sulindac normalized conduction velocity in caudal sensory fibers, sural myelinated fibers and sural unmyelinated fibers, and reduced the number of diabetic cataracts. Sulindac also normalized a deficit in dorsal root ganglion blood flow and a reduction in sciatic endoneurial oxygen tension in diabetic rats. Low dose insulin improved neuropathy as well but the pattern of benefits was less robust than that of sulindac. Sulindac may be a candidate for a clinical trial in human diabetic polyneuropathy.

Résumé: L'influence du sulindac sur la neuropathie diabétique expérimentale induite par le streptozotocin. Nous avons étudié l'influence du sulindac, un agent anti-inflammatoire non stéroïdien sur la neuropathie diabétique induite par le streptozotocin. Des rats diabétiques non traités ont été comparés à des rats non diabétiques, à des rats diabétiques traités avec de petites doses d'insuline et à des rats diabétiques traités au sulindac (6.0 mg/kg administré par gavage 5 jours sur 7). Nous avons évalué la neuropathie en suivant la conduction caudale motrice et sensitive sériée *in vivo*, la résistance à la défaillance de conduction d'origine ischémique et la conduction *in vitro* dans les fibres sensitives surales myélinisées et non myélinisées. L'impact de petites doses d'insuline et du traitement par le sulindac sur le micro-environnement du ganglion dorsal L4 et de l'endonèvre sciatique ont été évalués par la mesure de la perfusion locale et de la tension en oxygène à 16 semaines de diabète. Le sulindac a normalisé la vitesse de conduction das les fibres sensitives caudales, les fibres surales myélinisées et les fibres surales non myélinisées, et a réduite le nombre de cataractes diabétiques. Le sulindac a également normalisé un déficit du flot sanguin dans le ganglion dorsal et une réduction de la tension en oxygène dans l'endonèvre sciatique des rats diabétiques. L'insuline à faible dose a également amélioré la neuropathie, mais de façon moins marquée. Le sulindac pourrait être un candidat à un essai thérapeutique dans la polynévrite diabétique chez l'humain.

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Recent work has suggested that certain nonsteroidal anti-inflammatory agents may benefit models of experimental diabetic neuropathy. These preliminary studies could suggest an impact on human diabetic polyneuropathy. Of the agents tested, indomethacin[1] and piroxicam[2] have lessened indices of neuropathy in rats. At least two properties might account for these findings: inhibition of cyclo-oxygenase activity and aldose reductase inhibition. In diabetes, there are elevated levels of thromboxane but reduced prostacyclin. These features favour vasoconstriction and platelet aggregation and might be expected to aggravate microangiopathy.[3] Microangiopathy, and endoneurial hypoxia, in turn, are putative mechanisms of diabetic polyneuropathy.[4] Endoneurial hypoxia is observed in experimental diabetic neuropathy and human diabetic neuropathy, developing in the former as early as 4 weeks after the onset of hyperglycemia.[5-7] Moreover, oxygen treatment improves electrophysiological and axonal transport deficits in experimental diabetes[8-10] and hypoxic rearing of rats results in abnormalities that resemble diabetes.[11,12] Finally resistance to ischemic conduction failure, an important finding in diabetic neuropathy, appears particularly linked to hypoxia.[13,14]

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Sulindac is a substituted indene acetic acid chemically related to indomethacin that inhibits cyclo-oxygenase, platelet aggregation and aldose reductase.[15-17] These features render it an attractive choice in the treatment of diabetic neuropathy: it has been widely used in human arthritis with a known safety profile; it is long acting; and it has greater aldose reductase inhibitor (ARI) properties in human lens than indomethacin.[15-17] We studied the influence of sulindac on nerve conduction and tissue microenvironment in rats rendered diabetic by streptozotocin (STZ). To determine the mechanism of benefit of any treatment for experimental diabetic neuropathy ideally should include evaluation of its influence on endoneurial oxygen tension. This rigorous investigative approach, however, has rarely been employed. For example, aldose reductase inhibitors improve nerve conduction and endoneurial sorbitol levels but their impact on endoneurial oxygen tension is unknown.[18,19] Endoneurial blood flow has been studied more frequently but there are discrepancies among investigators as to whether it is truly reduced in experimental diabetes.[1,5,6,20-22] From previous work, we also have evidence that endoneurial hypoxia need not accompany endoneurial oligemia: these changes may occur independently.[1,6]

Also relevant toward the investigation of a potential therapy for diabetic neuropathy would be to evaluate the action of treatment on the dorsal root ganglion (DRG). Indeed, the prominence of sensory signs and symptoms in human diabetic polyneuropathy might suggest abnormalities in sensory ganglia. This aspect of treatment has not been previously examined in the experimental situation.

In this work we posed the following questions: (i) do electrophysiological improvements, in experimental diabetic neuropathy occur with insulin and sulindac and, if so, are they accompanied by improved endoneurial oxygen tension? (ii) are abnormalities of dorsal root ganglion oxygen tension or blood flow in experimental diabetes benefitted by insulin or sulindac treatment? Our work was based on the premise that the changes addressed in our model were relevant in the development of human diabetic polyneuropathy.

Materials and Methods

Animals

The experimental protocol was reviewed and approved by the local animal care committee to ensure ethical standards of treatment. Male Sprague-Dawley rats of initial weight 230-300 grams were injected with a single dose of intraperitoneal streptozotocin (STZ; Zanosar, Upjohn; 65 mg/kg) in citrate buffer at pH 4.5. Blood glucose measurements were made 5-7 days after injection and the rats were considered diabetic only if the values were equal to or higher than 16.0 mmol/L throughout the studies (measured at 1, 8 and 16 weeks following injection). Glucose measurements were made from the ventral caudal vein using a glucometer (AccuChek IIm; Boehringer Mannheim Canada; Dorval, Quebec) and the oxygen rate method (Beckman Glucose Analyzer 2; Beckman Instruments Inc.; Palo Alto, CA). The rats were housed in grouped wire floored cages with free access to rat chow and water. Cataracts were counted by visual inspection.

Four groups of rats were studied: (i) nondiabetic rats given citrate buffer without STZ at the onset of the studies; (ii) diabetic rats not given any specific form of treatment; (iii) diabetic rats given sulindac (6.0 mg/kg by gavage 5 of 7 days/week) starting 1 week after STZ injection; (iv) diabetic rats given low dose insulin (one unit of regular insulin each morning SQ starting 1 week after STZ). Sulindac was suspended at 6.0 mg/ml in a solution of 0.5 % methylcellulose and 0.2 % sodium benzoate. A low dose insulin regimen was deliberately chosen to reflect intermittent and limited control of hyperglycemia as frequently occurs in human diabetes.

Multifiber Nerve Conduction Recordings

Multifiber *in vivo* nerve conduction recordings were conducted 5-7 days after the injection of STZ or buffer and again 8 and 16 weeks later. Recordings were made in anesthetized rats (pentobarbital 65 mg/kg ip) and included measurements of sensory caudal (the nerve is mixed motor and sensory but conduction velocity is determined by the faster conducting sensory fibers) and motor caudal conduction. Technical details have been previously published.[1] Subcutaneous near nerve temperatures were maintained at 37 ± 1° C during the conduction measurements. Latencies were measured to the onset of the negative deflection of the potential and amplitudes calculated from baseline to peak (80 mm between the stimulating and recording electrodes for both the caudal motor and sensory measurements). Resistance to ischemic conduction failure (RICF) measurements were made by occluding the arterial supply of caudal mixed fibers with a proximal tail tourniquet inflated above arterial pressure – the time required for a 50% decline in the amplitude of the mixed nerve action potential (at a distance of 60 mm) was determined. *In vitro* recordings were made at the 16 week endpoint in sural myelinated and unmyelinated fibers in mineral oil, using a perspex chamber, at 37 ± 1° C, as previously described.[1] The sural nerves studied were taken from the leg opposite the site of sciatic endoneurial blood flow measurements and oxygen tension recordings.

Blood Flow, Microvascular Resistance and Oxygen Tension

The endpoint preparation was identical to that described in previous experiments.[1,6] Briefly, the rats were anaesthetized (pentobarbital 65 mg/kg ip), then underwent placement of a tracheostomy and left carotid arterial line to permit continuous recording of mean arterial pressure (MAP) throughout the experiment. For the microelectrode measurements the rats were paralyzed with tubocurarine (1.5 mg/kg intra-arterial then 0.8 mg/kg two hourly). Supplemental doses of pentobarbital were given approximately two hourly (20 mg/kg ip) to maintain a relatively constant level of anaesthesia (as judged by the level and stability of MAP). Only rats that maintained a consistent MAP throughout greater than 80 mmHg were deemed acceptable. Insulin and sulindac were witheld the day of surgery. Blood flow measurements were made on the left sciatic nerve (opposite the side where the sciatic and sural nerves were later removed for or *in vitro* conduction recordings respectively) or right L4 dorsal root ganglia (DRG). Techniques for sciatic endoneurial and DRG blood flow measurements have been previously described.[1,5,6,23] Not all animals had blood flow measurements and oxygen tension measurements in both structures, and those that did were randomly assigned to undergo either the DRG or sciatic endoneurial blood flow and oxygen tension measurements first. Blood flow was measured in the sciatic endoneurium and DRG using hydrogen clearance as previously described. Two hydrogen clearance curves were usually obtained from each structure and a mean blood flow measurement was calculated for each tissue. Endoneurial microvascular

resistance and dorsal root ganglion microvascular resistance were calculated as MAP/blood flow where MAP measured from the carotid arterial line was used to estimate perfusion pressure. Oxygen tension was measured in endoneurium or DRG (usually using the same microelectrode, but polarized to –0.65V) after the completion of the clearance curves and following bubbling of the mineral oil covering the nerve or DRG with 100% nitrogen, as in previous work:[1] 5 different depths from 2-3 separate electrode insertions yielded 10-15 measurements of $Pn0_2$ (endoneurium) or $Pdr0_2$ (DRG). The microelectrodes were then immediately calibrated in a water chamber with bubbled gas mixtures of 37° C at concentrations of 0, 10, and 25% to test for linearity and to determine $Pn0_2$ or $Pdr0_2$ from the calibration line. Experiments without a linear calibration line were discarded. $Pn0_2$ and $Pdr0_2$ histograms were constructed from pooled data from each animal group. In individual rats, values for mean oxygen tension, percentage of tensions below 15 torr and lowest recorded tension were determined for later comparisons among the treatment groups.

Data Analysis

Mean and standard error values were calculated for serial and endpoint electrophysiological studies, blood flow, MAP and microvascular resistance. The groups were compared using a one way analysis of variance with one-tailed Student's t tests used for post-ANOVA comparisons. The choice of the one-tailed test was justified by the expected direction of change in conduction, blood flow and oxygen tension from previous work by ourselves and others.[1,5,6,22]

Results

Diabetic rats gained less weight than nondiabetic or insulin-treated rats over the 16 week study. Hyperglycemia at endpoint was comparable among the groups (insulin was not given on the day of endpoint in the insulin-treated rats). There were fewer cataracts in the insulin or sulindac-treated rats than untreated diabetics. Results of final weights, glucose and inspection for cataracts are given in Table 1.

Table 1. Final Weights, Glucose, Cataracts.

Group (n*)	FINAL WEIGHT (grams)	FINAL GLUCOSE (mmol/L)	% CATARACTS PRESENT
Nondiabetics (25)	611 ± 10	6.5 ± 0.2	0%
Diabetics, untreated (29)	281 ± 10	24.6 ± 1.0	79%
Diabetics, insulin treated (12)	369 ± 14	22.3 ± 1.5	64%
Diabetics, sulindac treated (14)	319 ± 16	23.6 ± 1.5	57%

Table 2a. *In Vivo* Electrophysiological Results.

Group(n*)	1 Week	8 Weeks	16 Weeks
CAUDAL SENSORY (MIXED) FIBERS			
Conduction Velocity (sensory m/s)			
Nondiabetics (25)	39.6 ± 0.5[a]	50.4 ± 0.6[b]	54.2 ± 0.9[c]
Diabetics, untreated (29)	36.5 ± 0.6	45.8 ± 0.8	49.3 ± 1.0
Diabetics, insulin treated (12)	37.8 ± 0.6	48.7 ± 0.9	53.8 ± 1.5
Diabetics, Sulindac treated (14)	37.6 ± 0.6	47.5 ± 0.9	53.6 ± 1.5
Amplitude (mixed μV)			
Nondiabetics (25)	16.7 ± 0.9[d]	37.4 ± 2.0[e]	29.8 ± 1.5[+]
Diabetics, untreated (29)	13.3 ± 1.1	29.3 ± 1.5	27.2 ± 1.7
Diabetics, insulin treated (12)	20.5 ± 1.0	24.6 ± 1.7	35.9 ± 4.1
Nondiabetics Sulindac treated (14)	18.9 ± 1.2	24.9 ± 1.5	30.5 ± 2.2

Values are means ± SEM; ND-nondiabetic; D-untreated diabetic; DI-diabetes+insulin; DS-diabetes+sulindac
*number of rats at end point
+ ANOVA NS

a-p=0.001 (ANOVA); ND vs D, p=0.0001; DI, DS vs D, p=NS
b-p=0.0008 (ANOVA); ND vs D, p=0.0001; DI vs D, p=0.01; DS vs D, p=0.06
c-p=0.004 (ANOVA); ND vs D, p=0.0006; DI, DS vs D, p=0.01; DI, DS vs ND, p=NS
d-p=0.0001 (ANOVA); ND vs D, p=0.01
e-p<0.0001 (ANOVA); ND vs D, p=0.0005

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TABLE 2b. *In Vivo* Electrophysiological Results.

GROUP(n*)	1 WEEK	8 WEEKS	16 WEEKS
	CAUDAL MOTOR FIBERS		
Conduction Velocity m/s			
Nondiabetics (24)	31.4 ± 1.0+	41.2 ± 1.9+	45.3 ± 1.8f+
Diabetics, untreated (29)	27.5 ± 1.1	40.0 ± 1.7	41.5 ± 1.4
Diabetics, insulin treated (12)	30.6 ± 1.5	40.3 ± 1.3	42.7 ± 1.6
Diabetics, sulindac treated (14)	30.2 ± 1.5	41.8 ± 1.9	42.2 ± 1.8
Amplitude mV			
Nondiabetics (24)	3.62 ± 0.31g	5.06 ± 0.39h	4.23 ± 0.35i
Diabetics, untreated (29)	2.53 ± 0.29	3.34 ± 0.28	3.09 ± 0.25
Diabetics, insulin treated (12)	3.59 ± 0.37	3.75 ± 0.36	4.08 ± 0.46
Diabetics, sulindac treated (14)	4.04 ± 0.33	3.17 ± 0.33	3.89 ± 0.45

Values are means ± SEM; ND-nondiabetic; D-untreated diabetic; DI-diabetes+insulin; DS-diabetes+sulindac
*number of rats at end point
+ ANOVA NS

f-ND vs D, p=0.035
g-p=0.004 (ANOVA); ND vs D, p=0.006
h-p=0.0006 (ANOVA); ND vs D, p=0.0002
i-p=0.05 ANOVA; ND vs D, p=0.01; DI vs D, p=0.035; DS vs D, p=0.06

Table 2c. *In Vivo* Electrophysiological Results.

RESISTANCE TO ISCHEMIC CONDUCTION FAILURE (minutes)

GROUP(n*)	1 WEEK	8 WEEKS	16 WEEKS
Nondiabetics (23)	14.3 ± 0.5k	22.4 ± 0.6l	30.4 ± 0.7
Diabetics, untreated (27)	18.9 ± 0.7	29.5 ± 0.5	32.0 ± 0.8
Diabetics, insulin treated (12)	19.7 ± 0.7	28.3 ± 0.9	30.2 ± 1.3
Diabetics, sulindac treated (12)	20.7 ± 1.0	28.3 ± 0.9	29.7 ± 1.5

Values are means ± SEM; ND-nondiabetic; D-untreated diabetic; DI-diabetes+insulin; DS-diabetes+sulindac
*number of rats at end point
+ ANOVA NS

k-p<0.0001 (ANOVA); ND vs D, p<0.0001
l-p<0.0001 (ANOVA); ND vs D, p<0.0001

There were expected maturational rises in caudal sensory and motor amplitudes, conduction velocities and RICF in all of the groups studied, as noted before by ourselves and others.[1,6,24] As in previous work, sensory caudal conduction velocities were our most sensitive *in vivo* index of neuropathy-slowing in diabetics was noted as early as 1 week following STZ injection and was maintained thereafter at 8 and 16 weeks (Table 2a). Caudal motor conduction velocities were modestly reduced in diabetics, compared to nondiabetic controls at 16 weeks after STZ (Table 2b). Caudal compound motor action potential amplitudes were also reduced by diabetes at all time points studied (Table 2b). RICF was markedly prolonged in diabetics at 1 and 8 weeks after STZ but not at 16 weeks (a pattern also previously noted)[1] (Table 2c). In sural sensory fibers studied at endpoint (16 weeks) *in vitro*, both myelinated and unmyelinated conduction velocity was slowed in diabetics compared to nondiabetic controls (Figures 1A and 1B). The amplitude of the myelinated nerve action potential was also lowered by diabetes (Table 3).

Low dose insulin treatment improved *in vivo* sensory caudal conduction velocities at 8 and 16 weeks and the *in vitro* amplitude of the sural myelinated nerve action potential at 16 weeks (Table 3, Figure 1A). Sulindac improved sensory caudal conduction velocities at 16 weeks (there was borderline improvement at 8 weeks), and the *in vitro* sensory sural conduction velocities in both myelinated and unmyelinated fibers (Figures 1A, 1B).

Sciatic endoneurial blood flow and microvascular resistance were not influenced by diabetes, insulin or sulindac in comparison

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with nondiabetic controls, and were comparable to measurements from previous work in our laboratory.[1,6] In contrast DRG blood flow (GBF) was reduced by diabetes, but normalized by sulindac. Insulin treatment did not improve GBF. There was borderline higher DRG microvascular resistance in diabetic animals, compared to controls and to sulindac treated rats (4.13 ± 0.40 $mmHg.ml^{-1}.100g.min$ in nondiabetics vs. 5.09 ± 0.76 in untreated diabetics [p=0.1]; 3.76 ± 0.39 in sulindac treated diabetics [p=0.06 compared to nondiabetics]). Mean arterial pressure was lowered in all three diabetic groups and was not improved with treatment (nondiabetics - 127 ± 3; untreated diabetic - 115 ± 3; diabetic+insulin - 117 ± 2; diabetic+sulindac - 106 ± 3; p=0.002 ANOVA, nondiabetics vs untreated, insulin treated or sulindac treated, p<0.02). Results of blood flow measurements are given in Figure 2.

There was a lower mean $Pn0_2$ (sciatic endoneurium) in untreated diabetes, an increased percentage of $Pn0_2$ measurements less than 15 torr, and a reduction in the lowest $Pn0_2$ recorded among the multiple measurements of individual rats (Table 4, Figure 3A). Insulin corrected each of these parameters whereas sulindac reduced the percentage of $Pn0_2$ measurements less than 15 torr, and increased the lowest $Pn0_2$ recorded. Untreated diabetics had nonsignificant trends toward a reduction of the lowest $Pdr0_2$ (DRG) recorded, a lower mean $Pdr0_2$, and a higher percentage of measurements less than 15 torr (Table 4, Figure 3B).

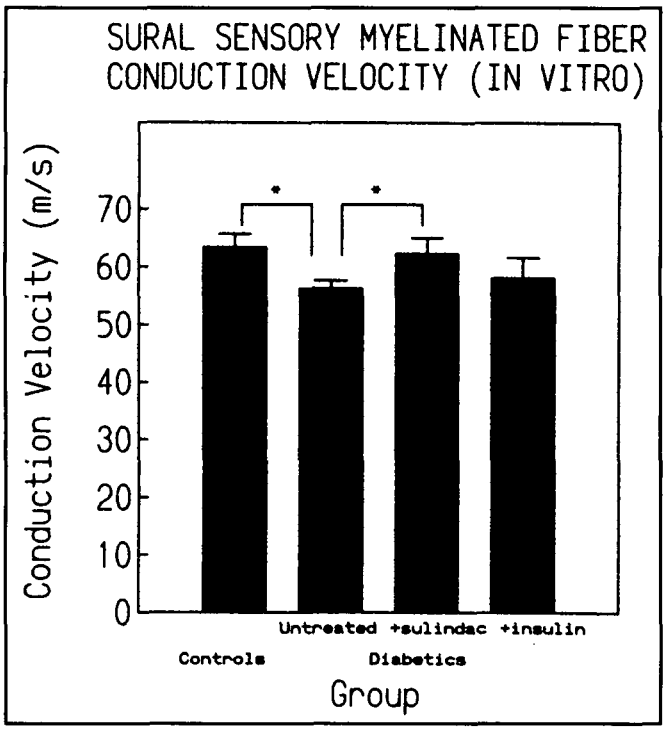

Figure 1A: Endpoint (16 weeks) in vitro *conduction velocity measurements in sural myelinated fibers. Sulindac normalized conduction velocity (nondiabetic vs untreated diabetics p=0.007; sulindac treated vs untreated p=0.03).*

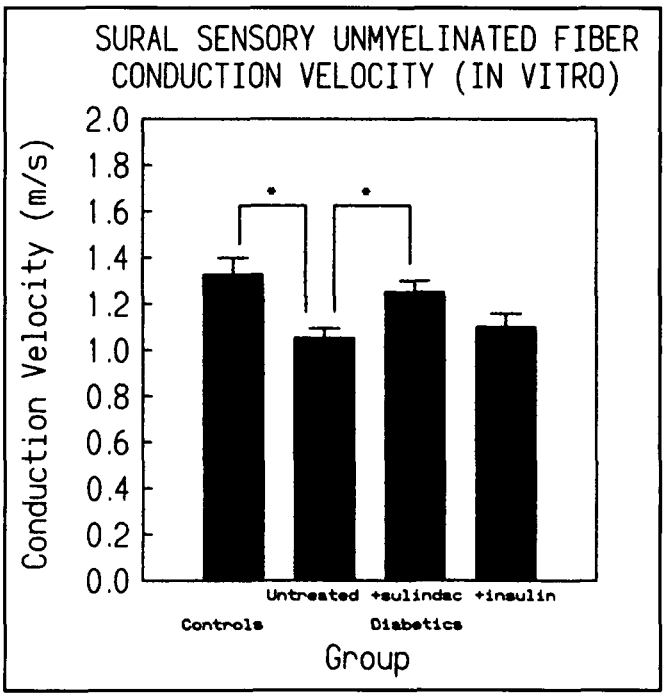

Figure 1B: Endpoint (16 weeks) in vitro *conduction velocity in sural unmyelinated fibers. Sulindac normalized conduction velocity (nondiabetic vs untreated diabetics p<0.001; sulindac treated vs untreated p=0.009).*

TABLE 3. *In Vitro* Sural Sensory Action Potentials.

GROUP (n)	MYELINATED FIBERS[a] (mV)	UNMYELINATED FIBERS (μV)
Nondiabetic	4.12 ± 0.60 (19)	63 ± 12 (14)
Diabetics, untreated	2.85 ± 0.27 (24)	58 ± 9 (19)
Diabetics, insulin treated	4.53 ± 0.48 (10)	96 ± 15 (9)
Diabetics, sulindac treated	3.63 ± 0.63 (13)	97 ± 18 (11)

Values are means ± SEM (ND, D), – see Table 2a
a→ND vs D; p=0.02

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Discussion

The electrophysiological features of neuropathy in this diabetic model were: (i) slowing of caudal sensory and sural sensory myelinated conduction velocity (ii) slowing of unmyelinated sural sensory conduction velocity; (iii) more mild changes in motor conduction velocity; (iv) an early and marked prolongation of RICF that was less prominent as the rat matured. In the sciatic endoneurium and DRG microenvironment: (i) diabetes was associated with lowering of oxygen tensions in the sciatic endoneurium and a similar but nonsignificant trend in DRG; (ii) there was a reduction of DRG blood flow in diabetic rats; (iii) insulin and sulindac both improved endoneurial oxygen tensions; sulindac improved caudal sensory, sural myelinated and sural unmyelinated conduction velocities whereas insulin's benefits were more modest; sulindac, but not insulin improved DRG blood flow; (iv) endoneurial blood flow was not altered by diabetes, or by treatment.

Sulindac's benefits were noted despite a similar deficit in maturational weight gain than untreated diabetics. The pattern of electrophysiological improvement was similar to that we noted previously with indomethacin,[1] whereas Cameron et al.[25] reported parallel changes with ponalrestat, an ARI. In their work, slowing in conduction velocity was greater in saphenous sensory fibers than several of the motor nerves tested and sensory fibers had the greatest response to treatment. Only partial responsiveness of motor fibers to an ARI was also reported by Yagihashi et al.[26] using ponalrestat. Thus, at least in the models where both motor and sensory conduction have been examined, sensory fibers are afflicted earlier by diabetes, but may respond better to aldose reductase inhibition. In our study, sulindac treated rats had fewer cataracts, a benefit that is suggestive of at least partial ARI action.

Sharma and Cotlier[16] reported the following potency of drugs in inhibiting human cataract aldose reductase: sulindac>indomethacin>salicylate>oxyphenbutazone. Yasuda et

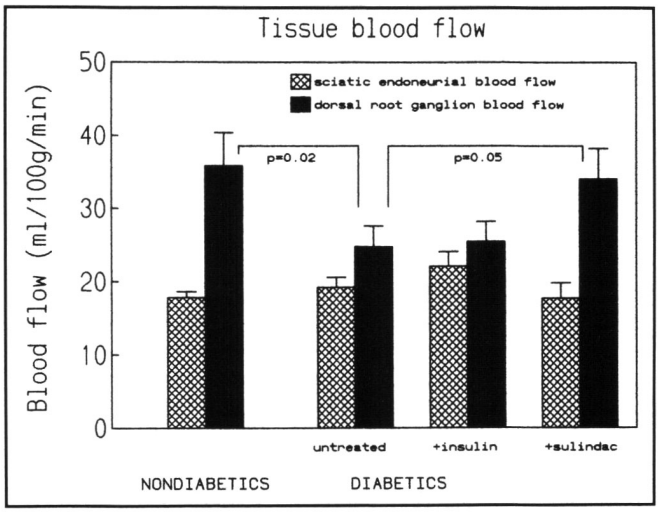

Figure 2: Sciatic endoneurial and lumbar dorsal root ganglion blood flow measurements made by hydrogen clearance polarography. Sciatic endoneurial blood flow did not differ among the groups. Dorsal root ganglion blood flow was significantly higher in controls or diabetics treated with sulindac than untreated diabetics. Nondiabetics n = 6 in sciatic, n = 11 in DRG; Untreated diabetics n = 19 in sciatic, n = 14 in DRG; Diabetic + insulin n = 11 in sciatic, n = 9 in DRG; diabetic + sulindac n = 10 in sciatic, n = 11 in DRG.

Table 4. Oxygen tensions.

	CONTROLS	DIABETICS		
		untreated	+insulin	+sulindac
Dorsal root ganglion				
Mean $PdrO_2$ (torr) (n)	57 ± 6 (15)	45 ± 7 (8)	72 ± 9 (7)	32 ± 7 (8)
% $PdrO_2$<15 torr	7 ± 4	11 ± 7	7 ± 7	14 ± 9
Lowest torr recorded	41 ± 7	26 ± 8	62 ± 10	26 ± 7
Sciatic endoneurium				
Mean PnO_2 (torr[a]) (n)	71 ± 8 (6)	39 ± 7 (10)	62 ± 6 (11)	49 ± 5 (10)
% PnO_2<15 torr[b]	0	36 ± 12	0	2 ± 2
Lowest torr recorded[c]	48 ± 10	14 ± 6	48 ± 5	32 ± 4
Arterial blood				
PO_2 (torr)	151 ± 5	161 ± 4	128 ± 4	150 ± 7
PCO_2 (torr)	40 ± 1	41 ± 1	41 ± 1	40 ± 1
pH	7.35 ± 0.01	7.38 ± 0.01	7.38 ± 0.01	7.39 ± 0.01

Values are means ± SEM (ND, D, DI, DS – see Table 2)
n value refers to number of rats tested (on average 10 tension measurements were made/rat
a → p=0.01 (ANOVA); ND, or DI vs D p<0.01
b → p=0.0006 (ANOVA); ND, or DI, DS vs D p<0.002
c→ p=0.0005 (ANOVA); ND or DI, DS vs D p<0.02

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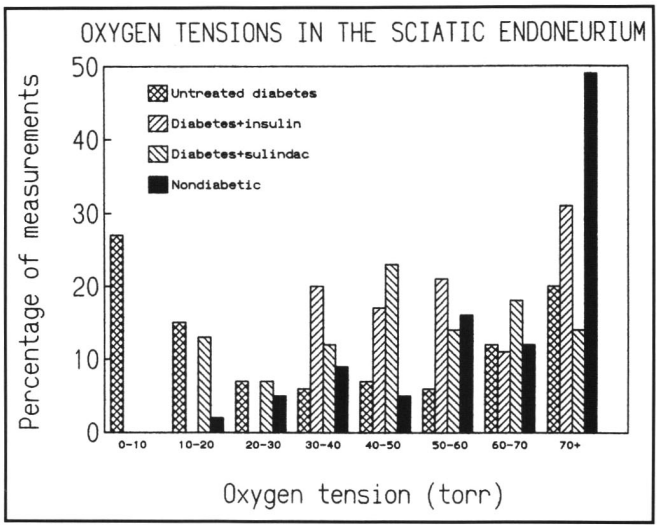

Figure 3A: *Oxygen tension histogram from individual measurements of oxygen tension in the sciatic endoneurium. Each step represents the percentage of measurements within a 10 torr envelope. Note that untreated diabetics have a large proportion of values in the lower oxygen tension ranges, as compared to the other groups. [Nondiabetic controls, number of measurements = 57, untreated diabetics = 82, sulindac + diabetes = 101, insulin + diabetes = 95].*

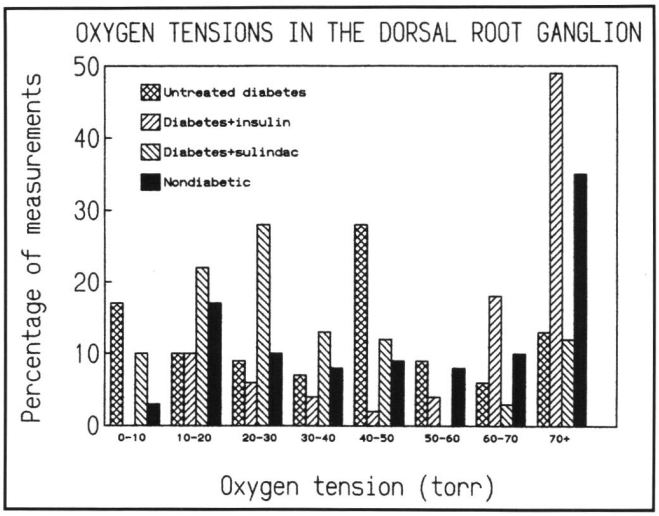

Figure 3B: *Oxygen tension histogram from individual measurements of oxygen tension in the lumbar dorsal root ganglion. Each step represents the percentage of measurements within a 10 torr envelope. Values are shifted toward a lower range by diabetes but the change is less striking than in the endoneurium, [Nondiabetic controls, number of measurements = 118, untreated diabetics = 69, insulin + diabetes = 50, sulindac + diabetes = 60].*

al.[27] recently concluded, however, that combining treatment with an ARI with a prostaglandin E1 analogue (directed toward treatment of microangiopathy) might offer advantages over ARI's. Sulindac's combined properties with possible ARI activity and cyclo-oxygenase inhibition could provide more than one benefit as a single agent. To fully explore the possible ARI action of sulindac in the present work, would require measurements of nerve sorbitol. In separate work, however, sulindac was not effective in reducing nerve sorbitol with 1 month of treatment (Zochodne et al., unpublished data).

The changes in both endoneurium and DRG microenvironment in untreated diabetes or following its treatment are complex and not easily explained by single mechanisms. As in previous work,[1,6] they reinforce our suggestion that local tissue blood flow, as determined by hydrogen clearance, and measurements of local oxygen tension do not necessarily change in tandem. Although local clearance of hydrogen may reflect, to some degree, the pattern of oxygen delivery, there are a number of reasons why these measurements may operate independently. For example, plasma clearance of hydrogen need not require transcapillary passage of erythrocytes that carry oxygen. Measurements of regional hematocrit, reflecting local microvascular viscosity, differ considerably from aortic hematocrit such that rises in local hematocrit could be associated with altered transcapillary transit times and reduced oxygen delivery.[28] There may be "skimming" at microvascular bifurcations in diabetes, with less passage of erythrocytes to the nutritive capillary circulation, as occurs at bifurcations in normal microvessels.[29] Loss of erythrocyte deformability is also a feature of diabetes, and interestingly, may be correctable by ARIs.[30,31] Similarly, the release of oxygen from hemoglobin may be altered by diabetes, although previous work has suggested that increased levels of 2,3 DPG compensate for this deficit.[32] In DRG, unlike endoneurium, metabolic demands are higher but could be downregulated by lesser oxygen availability.[23,33,34] Lower DRG oxygen consumption might then compensate for reduced delivery, yielding changes in blood flow that do not accompany changes in oxygen tensions.

In addition to the above changes in oxygen delivery and consumption, there is considerable evidence that diabetes is associated with "functional", if not overt microangiopathy. Indeed vasoreactivity in diabetic macrovessels and microvessels is altered but a major problem is deciding whether these changes in peripheral nerve induce electrophysiological abnormalities or are simply associated developments.[35-40] In this work, and previous work, we have not confirmed that experimental diabetic neuropathy is associated with endoneurial oligemia, as reported by others.[1,5,6,22] It is difficult to ascribe reductions in nerve blood flow to significant structural microangiopathy after only one week of diabetes, as reported by Cameron et al.[22] It is possible that some of these changes, associated with more severe hyperglycemia than our work, could arise from acute capillary plugging by nondeformable erythrocytes (also explaining their improvement with guanethidine, an agent that dilates endoneurial microvessels). Indeed, Bareford et al.[41] noted that erthrocyte deformability particularly declined with very high glucose levels, as the case in some of the reports of oligemia in experimental diabetes. Normal or increased sciatic blood flow has also been reported by others[20,21] whereas endoneurial blood vessels in diabetes are unremarkable or paradoxically have increased luminal areas.[42,43]

It is tempting to speculate that sulindac's improvement in doral root ganglion blood flow accounted for its benefits to sensory conduction. ARIs reduce basement membrane thickening of microvessels in retina and could account for the improvement in DRG blood flow by a similar inhibition of microvessel damage.[44] Alternatively, and perhaps more likely sulindac may have improved flow by reducing platelet aggregation through inhibition of cyclo-oxygenase.[15]

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To address complex questions of pathogenenesis may require a great deal of further work. For validity, large numbers of oxygen tension measurements are required to construct histograms that reflect a given tissue microenvironment. It would be necessary to study oxygen tension and blood flow in dorsal root ganglia of diabetic animals in serial fashion to determine what the exact relationship of these deficits would be over time. Secondly, it would then be important to compare the following interventions at each time point: high dose insulin treatment with euglycemia; low dose insulin; "selective" aldose reductase inhibition that is effective in nerve; and "selective" cyclo-oxygenase inhibition.

Sulindac has a well recognized safety profile in humans, and is currently employed as an anti-arthritic agent. The major limitations that would have to be considered in a human trial of sulindac are the gastropathy and nephropathy reported with this class of drug, but these side effects have not seriously restricted its clinical use.[45] Sulindac's influence on diabetic nephropathy would also have to be carefully considered.

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