Dorsal root ganglia microenvironment of female BB Wistar diabetic rats with mild neuropathy

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Abstract

Abnormalities in the microenvironment of dorsal root ganglia (DRG) might play a role in the pathogenesis of sensory abnormalities in human diabetic neuropathy. We examined aspects of DRG microenvironment by measuring local blood flow and oxygen tension in the L4 dorsal root ganglia of female BB Wistar (BBW) diabetic rats with mild neuropathy. The findings were compared with concurrent measurements of local sciatic endoneurial blood flow and oxygen tension. Diabetic rats were treated with insulin and underwent electrophysiological, blood flow and oxygen tension measurements at either 7–11 or 17–23 weeks after the development of glycosuria. Nondiabetic female BB Wistar rats from the same colony served as controls. At both ages, BBW diabetic rats had significant abnormalities in sensory, but not motor conduction compared to nondiabetic controls. Sciatic endoneurial blood flow in the diabetic rats of both ages was similar to control values, but the older (17–23 week diabetic) BBW diabetic rats had a selective reduction in DRG blood flow. Sciatic endoneurial oxygen tensions were not significantly altered in the diabetic rats. DRG oxygen tension appeared lowered in younger (7–11 week diabetic) but not older (17–23 week diabetic) BBW rats. Our findings indicate that there are important changes in the DRG microenvironment of diabetic rats with selective sensory neuropathy.

Keywords: Diabetic neuropathy; Blood flow; Oxygen tension; BBW diabetic; Dorsal root ganglion

1. Introduction

Abnormalities in the microenvironment of dorsal root ganglia (DRG) have not been evaluated in the context of experimental diabetic neuropathy. Early and prominent sensory dysfunction is important in human diabetic subjects (Brown and Asbury 1984; Dyck et al. 1987), but DRG have not been considered relevant in this selectivity. This is puzzling because DRG have greater metabolic requirements than endoneurium with higher blood flow and lower oxygen tension (Greene et al. 1979; Kadekaro et al. 1985; Zochodne and Ho 1991). DRG might thus be particularly vulnerable to diabetic microangiopathy. In the sciatic endoneurium, lowered endoneurial oxygen tension has been a constant finding in STZ diabetic rats (Tuck et al. 1984; Zochodne and Ho 1992a,b). Sural nerve oxygen tensions are also lowered in human diabetes (Newrick et al. 1986). Lowered blood flow in the endoneurium of rats with experimental diabetes, however, has been found by some, but not by other investigators (Tuck et al. 1984; Pugliese et al. 1989; Cameron et al. 1991; Zochodne and Ho 1992a,b). The discrepancy is important because the presence of both hypoxia and oligemia in nerve suggest a role of early microangiopathy in the pathogenesis of the disease. In contrast, hypoxia exclusive of oligemia might arise from mechanisms other than microangiopathy.

Most experimental studies of blood flow and oxygen tension in the diabetic rat model have evaluated STZ-induced diabetes. In this model, motor conduction abnormalities have been regarded as the benchmark electrophysiological index of neuropathy whereas sensory changes have only been reported in some instances. Also, insulin treatment has often not been applied. A relevant animal model of human diabetes might thus be one in which sensory abnormalities ex-
ceed motor changes, where the onset of diabetes is spontaneous and where insulin treatment is applied. The BB Wistar model of spontaneous inherited diabetes is suited to fit these requirements (Sima et al. 1987). Insulin treatment is required for animal survival, and may render more mild neuropathy from better control of hyperglycemia than untreated STZ-injected rats. In studies reported here, this more mild neuropathy was manifest as abnormalities in sensory, but not motor fibers, raising the possibility that the DRG might be a relevant site of pathology.

We pose the following questions in this work: (i) what are the electrophysiological features of motor and sensory fibers in insulin-treated female BBW diabetes? (ii) Is BBW neuropathy associated with endoneurial hypoxia or ischemia? (iii) Are there alterations in the DRG microenvironment of BBW diabetic rats that accompany neuropathy? To address these questions, we studied female BBW diabetic rats at 7-11 or 17-23 weeks of diabetes and compared both motor and sensory conduction with nondiabetic controls from the same colony. Tissue measurements of blood flow were made using hydrogen clearance and oxygen tension histograms constructed from multiple measurements made with oxygen-sensitive microelectrodes.

2. Methods

Animals

The experimental protocol was reviewed and approved by an Animal Care Committee to ensure ethical standards of treatment.

BB Wistar female diabetic rats were made available to us by the Health Protection Branch in Ottawa (Canada) and transferred to Queen's University, Kingston, prior to endpoint. These rats were housed in plastic shoebox cages covered with wood chips or shavings with free access to rat chow (Rodent Laboratory Chow #5001; Ralston Purina, Woodstock, Canada) and water. Diabetes was detected by the onset of glycosuria and the rats were then started on insulin using a sliding scale dosage regimen: 2.0 units of protamine zinc insulin SQ (or NPH insulin) at the onset of glycosuria, increased by 0.2 units if there was a persistent loss of body weight and further glycosuria. The mean time of onset of glycosuria after birth was $15 \pm 1$ weeks but varied in individual rats (Table 1). The age of the rats, and the duration of their diabetes is given in Table 1. From the same colony of rats, nondiabetic female Wistar rats served as controls.

Electrophysiological recordings, blood flow measurements, and oxygen tension measurements were made during overlapping time frames by a single technician (LH) to ensure uniformity of technique.

Multifiber nerve conduction recordings

Multifiber electrophysiologic recordings were made at endpoint, 7–11 weeks after the first detection of glycosuria in the younger group (approximately 25 weeks of age) and 17–23 weeks after the first detection of glycosuria in the second older group (approximately 33 weeks of age). Final studies in control rats were at an approximate age of 30 weeks. All recordings were made in anesthetized rats (pentobarbital 65 mg/kg) and included measurements of sensory caudal (the nerve is mixed motor and sensory but conduction velocity is determined by the faster conducting sensory fibers) motor caudal conduction and sciatic-tibial motor conduction. Technical details have been previously published (Zochodne and Ho 1992b). Subcutaneous near nerve temperatures were maintained at $37 \pm 1^\circ$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 in both caudal nerves and from sciatic notch stimulation in sciatic-tibial fibers). Resistance to ischemic conduction failure (RICF) measurements were made in rats 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.

Blood flow, microvascular resistance and oxygen tension

The endpoint preparation was identical to that described in previous experiments (Zochodne and Ho 1992a,b). Briefly, the rats were anesthetized (pento-
barbital 65 mg/kg i.p.), 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 2 hourly). Supplemental doses of pentobarbital were given approximately 2 hourly (20 mg/kg) to maintain a relatively constant level of anaesthesia (as judged by the level and stability of the MAP). Only rats that maintained a consistent MAP throughout of > 80 mm Hg were deemed acceptable. Insulin was withheld the day of surgery in BBW rats. Blood flow measurements were made on the left sciatic nerve or right L4 dorsal root ganglia (DRG) and were routinely calculated from the slow component of biexponential clearance curves, or the single component of monoexponential curves (see Zochodne and Ho 1991, 1992a,b). Not all animals had blood flow measurements and oxygen tension measurements in both structures, and those that did were randomly assigned to undergo either DRG or sciatic studies first. Blood flow was measured in the sciatic endoneurium and DRG using hydrogen clearance as previously described (Zochodne and Ho 1991, 1992a,b). Two hydrogen clearance curves were usually obtained from each structure and a mean blood flow measurement was calculated for each tissue. Oxygen tension was measured in endoneurium or DRG (usually using the same microelectrode, but polarized to -0.65 V) 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 (Zochodne and Ho 1991, 1992a,b): 5 different depths from 2–3 separate electrode insertions yielded 10–15 measurements of PnO₂ (endoneurium) or PdO₂ (DRG). The microelectrodes were then immediately calibrated with a bubbled gas mixture at 37°C at concentrations of 0, 10, and 25% to test for linearity and to determine PnO₂ or PdO₂ from the calibration line. Experiments without a linear calibration line were discarded. PnO₂ and PdO₂ histograms were constructed from pooled data from each animal group. To avoid drift, the microelectrodes were left in situ within endoneurium to stabilize before the oxygen tensions were recorded. We have compared pre and post endoneurial insertion calibration and have not noted any significant advantage in pre-insertional calibration. In controls, we have routinely observed some non-gaussian skew in the histogram with a peak in the > 60 Torr range possibly from atmospheric diffusion or inadvertent arteriolar sampling. Microvascular resistance was calculated as MAP/blood flow.

The choice of endpoint time was justified by changes observed at these times by work in our laboratory (Zochodne and Ho 1992a,b) and that of others (Tuck et al. 1984).

Data analysis

Mean and SE values were calculated for serial and endpoint electrophysiological studies, blood flow, MAP and microvascular resistance. Statistical comparisons among the three groups were made using a one-way analysis of variance (ANOVA) and post-ANOVA Student’s t-tests. Oxygen tensions were compared among the groups by constructing histograms from pooled individual measurements whereas mean tensions and related statistics were analyzed in individual animals before determining group mean values.

Oxygen tension histograms were constructed from the following numbers of individual measurements: BBW diabetic (7–11 weeks), 56 (6 rats) in DRG and 42 (6 rats) in sciatic endoneurium; BBW diabetic (17–23 weeks), 51 (7 rats) in DRG and 46 (5 rats) in sciatic endoneurium; BBW nondiabetic controls, 70 (7 rats) in DRG and 70 (6 rats) in sciatic endoneurium.

3. Results

Hyperglycemia was confirmed at endpoint by withholding insulin for 24 hours before the final studies. The glucose levels after insulin withdrawal and endpoint glycated hemoglobin levels are given in Table 1.

In BBW rats of 7–11 weeks diabetes duration, there was a significant slowing of caudal sensory conduction velocity and a reduction in the amplitude of the compound nerve action potential from caudal fibers (Table 2). BBW rats of 17–23 weeks diabetes duration had similar abnormalities in sensory caudal conduction.

Both diabetic groups had only a trend (not statistically significant) toward slowing of conduction velocity in sciatic tibial and caudal motor fibers. Sciatic-tibial mo-

<table>
<thead>
<tr>
<th>Table 2</th>
<th>In vivo electrophysiological results in BB Wistar Rats at endpoint. Results are means ± SEM</th>
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<tbody>
<tr>
<td></td>
<td>Diabetics</td>
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<tr>
<td></td>
<td>Young</td>
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<td></td>
<td>(17–23 weeks)</td>
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<tr>
<td>Sensory caudal</td>
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<td>$n$</td>
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<tr>
<td>CV (m/s)</td>
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<td>CV (m/s)</td>
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<td>Amp (mV)</td>
<td>4.50 ± 0.46</td>
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<tr>
<td>Motor sciatic-tibial</td>
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<tr>
<td>CV (m/s)</td>
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<td>Amp (mV)</td>
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<tr>
<td>RICF</td>
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$a$ ANOVA NS.
$b$ $p < 0.0001$ (ANOVA); Young, older vs controls $p < 0.001$.
$c$ $p < 0.0001$ (ANOVA); Young vs older controls $p < 0.001$; young vs controls $p = 0.01$. 

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Dorsal root ganglion

<table>
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<th>Diabetics (n)</th>
<th>Controls (n)</th>
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<tr>
<td></td>
<td>BBW (7–11)</td>
<td>BBW (17–23)</td>
</tr>
<tr>
<td>MR (mm Hg·ml⁻¹·100 g⁻min)</td>
<td>4.58 ± 0.60 (8)</td>
<td>5.49 ± 0.60 (10)</td>
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<td>MAP (mm Hg)</td>
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<td>109 ± 4</td>
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Sciatic endoneurium

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<th>Diabetics (n)</th>
<th>Controls (n)</th>
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<tr>
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<td>BBW (7–11)</td>
<td>BBW (17–23)</td>
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<tr>
<td>MR (mm Hg·ml⁻¹·100 g⁻min)</td>
<td>6.55 ± 0.51 (9)</td>
<td>5.81 ± 0.53 (7)</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>107 ± 5</td>
<td>113 ± 8</td>
</tr>
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</table>

* ANOVA NS.

a BBW (17–23) vs controls p = 0.035.

b > p = 0.012 (ANOVA); diabetics vs controls p < 0.03.

Tor potentials were reduced in amplitude in diabetic rats. RICF was not significantly prolonged.

Results of blood flow measurements are given in Fig. 1. Microvascular resistance and mean arterial pressure are shown in Table 3.

In the younger 7–11 week BBW rats, DRG blood flow was similar to measurements made in BBW nondiabetic control rats and was not statistically different than matched historical values of normal rats from our laboratory. At 17–23 weeks, however, DRG blood flow in BBW rats was significantly lower than BBW nondiabetic control measurements, and historical normal DRG blood flow measurements from our laboratory. Microvascular resistance was normal in younger (7–11 week) BBW DRG but elevated in the DRG of older BBW diabetic rats. In contrast, sciatic endoneurial blood flow and microvascular resistance were comparable to controls, irrespective of the duration of diabetes.

MAP taken during the sciatic endoneurial blood flow measurements was lower in both diabetic groups than nondiabetic controls. These differences in MAP were not observed in the DRG studies, possibly because of the greater invasiveness of the DRG experiment and the acceptance only of studies with a MAP ≥ 80 mm Hg.

Oxygen tensions had a non-gaussian distribution despite the large sample size used to construct the histogram. We have previously observed this feature and suspect it reflects the nonhomogenous structure of the tissues being examined (Zochodne and Ho 1992a,b).

The oxygen tension histogram (Fig. 2), was shifted to lower values in the DRG of young (7–11 week) BBW diabetic rats, manifest as an increase in the percentage of measurements < 15 Torr (Table 4). This reduction was present despite slightly higher arterial oxygen tensions, results that could slightly increase tissue values.
SCIENTIFIC ENDONEURIAL AND DORSAL ROOT GANGLION BLOOD FLOW

Fig. 1. Dorsal root ganglion and sciatic endoneurial local blood flow in diabetic and control animals. The numbers below each histobar give the number of weeks each group was diabetic. Historical controls (LAB CON) were derived from the same laboratory in control Sprague-Dawley rats using identical measurement techniques (see Methods). The p values reflect comparisons of dorsal root ganglion blood flow among the groups indicated. Sciatic endoneurial blood flow did not differ among the groups.

recorded. No definite shift in DRG oxygen tension was present in the older BBW (17-23 week) diabetic rats. Sciatic endoneurial oxygen tension was similar to non-diabetic control values in both BBW diabetic groups (Fig. 3, Table 4).

4. Discussion

The major findings of this work were: (i) insulin-treated female BBW diabetic rats in this model developed mild neuropathy without significant slowing of motor conduction velocity; (ii) there were significant reductions of sensory caudal conduction velocity and the amplitudes of the caudal nerve action potentials;

(iii) sciatic endoneurial blood flow, microvascular resistance and oxygen tensions were normal in the diabetic rats; (iv) older diabetic BBW rats had a reduction in DRG blood flow; (v) younger diabetic BBW rats had lowered DRG oxygen tension.

It was also important to note that our diabetic rats had a comparable weight to nondiabetic controls at endpoint. This may reflect their insulin treatment, only mildly elevated glycated hemoglobin values and the use of female, rather than male rats. Many previous studies using STZ-induced diabetes have been confounded by dramatic differences in weight between diabetics and controls. These differences, in turn, make it more difficult to interpret changes in electrophysiological studies and tissue microenvironment. Our controls had ages between the two diabetic groups such that the difference between controls and the older diabetics was inconsequential. These differences do not account for our findings.

It is possible that the trends toward slowing of motor conduction and prolongation of RICF would have been statistically significant had we applied insulin in lower doses or extended the duration of our protocol. Our choice of endpoint, however, was based upon previous STZ work where significant sciatic oligemia and hypoxia occurred at 16 weeks following hyperglycemia (Tuck et al. 1984). The more mild neuropathy in our model, where sensory abnormalities exceeded motor findings may better model human disease. In our model, the only significant alterations in peripheral nerve tissue microenvironment were in dorsal root ganglia and these accompanied sensory electrophysiological abnormalities. In contrast, normal sciatic-tibial motor conduction velocities was associated with unaltered sciatic endoneurial oxygen tension. We do not regard the reduced amplitude of the sciatic-tibial motor potentials as reliable evidence of neuropathy since they may reflect direct diabetic changes in muscle
or technical factors (e.g. subcutaneous fat at the site of the recording electrodes might alter the results selectively). The findings may argue that significant motor fiber abnormalities require the development of endoneurial hypoxia. RICF was also unchanged in our model, and has been more directly linked to endoneurial hypoxia (Low et al. 1986). It is worthwhile, however, indicating that neither the present work nor other investigations examining local oxygen tension and blood flow in diabetic nerve have proven, with serial studies, that hypoxia or ischemia predate nerve conduction abnormalities.

Previous work in our laboratory with overt diabetic neuropathy in STZ-injected male rats failed to identify a delay in sciatic endoneurial hydrogen clearance that would indicate oligemia (Zochodne and Ho 1992a,b). These normal blood flow measurements were observed despite the finding of reduced endoneurial oxygen tension. To explain these findings, we have argued that deficits in oxygen delivery in diabetic nerve might be independent of actual plasma flux, a major component of hydrogen clearance. At normal microvascular bifurcations for example, plasma flow and oxygen-carrying RBC flow may stream differently (Pries et al. 1989). This could explain why other workers using reliable techniques have consistently failed to identify changes in endoneurial blood flow in experimental diabetes and why histological studies of diabetic endoneurial microvessels have been unremarkable (Sharma and Thomas 1974; Pugliese et al. 1989). Indeed, the identification of endoneurial oligemia as early as 1 week after the induction of severe STZ diabetes in one study argues against significant microangiopathy in early diabetes but may suggest that there may be hyperglycemia-induced microvesSEL plugging, perhaps by nondeformable RBCs (Cameron et al. 1991; Rillaerts et al. 1988; Simpson 1988; Kowluru et al. 1989). We cannot fully exclude an acute alteration in the microenvironment that occurred from withholding insulin at endpoint for 24 h (although both younger and older rats would have been equally susceptible).

It is not surprising then, in the present work, to observe a significant discrepancy between measurements of hydrogen clearance in DRG and their respective oxygen tensions. In DRG there could also be marked reductions in perikaryal oxygen consumption, especially later in the disease, that would impact on the oxygen tensions measured. This could explain our finding of DRG oligemia but essentially normal oxygen tension in the older BBW diabetic rats. The DRG findings in the younger BBW rats might occur because of a deficit in oxygen delivery or excessive oxygen consumption with lowering of measured tensions but preserved plasma flux as measured by hydrogen clearance. In summary, in earlier diabetes there may be alterations in RBC transcapillary transit from hyperglycemia resulting in tissue hypoxia; DRG may be more susceptible to these changes than the endoneurium because of their metabolic requirements and the relatively large size of endoneurial capillaries (Bell et al. 1984); in later disease of moderate hyperglycemia and insulin treatment, oxygen consumption in DRG may decline either as a result of lowered blood flow or may result in a secondary decline in blood flow; in severe hyperglycemia of some STZ models, there may be capillary plugging such that hydrogen clearance is delayed in endoneurium as well. Direct measurements of high energy substrates in DRG would help address the possibility of significant DRG ischemia.

Our discrepant findings in DRG microenvironment could also simply arise from random experimental error and the inherent difficulties in estimating mean tissue oxygen tensions with microelectrodes in a given tissue space. To reduce this possibility, we employed a large number of repeated measurements of oxygen tension at different depths and calibrated the microelectrodes in a tissue bath immediately after each set of measurements. The post-insertional calibration technique offers results as reliable or perhaps better than pre and post insertional calibrations since protein contact with the platinum microelectrode within nerve might reduce its sensitivity. This would render pre-insertional values calibration too high, underestimating results obtained. It is possible that a direct measurement of RBC, rather than plasma flux, as offered by the laser doppler flowmeter, could address some of these discrepancies, if selective endoneurial or intracapsular measurements could be made. Unfortunately laser doppler measurements are not quantitative and are heavily influenced by RBC flux of extrinsic non-nutritive blood vessels. In the mixed peripheral nerve trunk there are no obvious structural distinctions between myelinated motor and sensory fibers that would explain the difference in their susceptibility to diabetes. Dorsal root sensory fibers had greater susceptibility to hypoxia and hyperglycemia than ventral root motor fibers in one study but these findings do not address questions of pathogenesis in the mixed nerve trunk (Schneider et al. 1992). Endoneurial hypoxia and oligemia or sorbitol accumulation might be associated with deficits in both fiber classes. The DRG, however, does not possess a “tight” blood-nerve barrier, as in endoneurium, and might be more likely to accumulate sorbitol or have its structural proteins glycosylated (Arvidson 1979; Ohi et al. 1985; Brownlee et al. 1988). We did not examine these possibilities in this work. DRG, however, have higher metabolic demands than the nerve trunk and we have previously reported that higher blood flow, evidence of partial autoregulation and lower tissue oxygen tension values in DRG reflect these demands (Greene et al. 1979; Kadekaro et al. 1985; Zochodne and Ho 1991). Further lowering of
oxygen tension or loss of autoregulation might have a more deleterious influence on this structure. It is not known whether these changes might then result in a defect of neurofilament synthesis and transport leading to selective sensory fiber atrophy.

Although technically demanding, it would be important to clarify the relationships among the duration and degree of hyperglycemia, electrophysiological abnormalities and alterations in microenvironment. The BBW rat may be the best model to accomplish this.

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