Diabetes mellitus prevents capsaicin from inducing hyperaemia in the rat sciatic nerve

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Summary. Loss of neurogenic inflammation in response to tissue injury may be an important complication of diabetes mellitus. We studied local neurogenic inflammation in the peripheral nerve trunk of Sprague-Dawley rats 4 months following the induction of diabetes by streptozotocin injection. To assess neurogenic inflammation, the epineurial plexus of the sciatic nerve was exposed to topical capsaicin, an agent that releases vasoactive neuropeptides from perivascular afferent terminals. Under normal circumstances, local vasodilatation results in endoneurial hyperaemia or a 'flare'. We evaluated the influence of capsaicin in diabetic sciatic nerve by making serial measurements of endoneurial blood flow using microelectrodes sensitive to hydrogen clearance. After 4 months of hyperglycaemia (glucose > 16.0 mmol/l), diabetic animals had slowing of unmyelinated and myeli-

nated sural sensory conduction velocity compared to citrate buffer injected controls. Baseline sciatic endoneurial blood flow was unaltered by diabetes, and was comparable to controls. There was an expected hyperaemic response of endoneurial blood flow to capsaicin in control rat sciatic endoneurium but no consistent 'flare' response in diabetic rats. Our findings indicate that there is loss of capsaicin-related neurogenic inflammation in the vasa nervorum of experimental diabetes. It is possible that a similar deficit following nerve injury could impair the milieu for axonal regeneration in diabetes.

Key words: Diabetic neuropathy, vasa nervorum, neurogenic inflammation.

In skin and other tissues, there is evidence that diabetes impairs microcirculatory responses to injury, a feature that may contribute toward poor healing [1]. In particular, an important response to injury is the development of neurogenic inflammation, in which afferent nerve fibres release local peptides that increase local blood flow, allow plasma extravasation, and recruit immunomodulatory cells [2]. Substance P (SP), calcitonin gene-related peptide (CGRP), neurokinin A and neurokinin B are among the nerve terminal peptides that participate in these local responses [3]. SP and CGRP, in concert with histamine that they help release from local mast cells, are local vasodilators of microvessels, probably acting through nitric oxide [4–7]. In diabetes, the local neurogenic flare response, perhaps mediated by an 'axon reflex' may be impaired [8, 9].

Diabetic peripheral nerve recovers slowly following focal injury but the factors that contribute toward impaired regeneration are not understood. Aldose reductase inhibitors correct metabolic abnormalities and improve nerve conduction but fail to improve regeneration in diabetic nerves [10]. Hypoxia and reduced local blood

flow have been observed in uninjured nerves of animals with experimental diabetic neuropathy and could contribute poor recovery [11, 12]. Not all reports, however, have identified these reductions in blood flow [13–15].

A model of nerve trunk 'neurogenic inflammation' is that associated with the acute action of capsaicin, an agent that depolarizes peptidergic afferent fibres, including perivascular terminals [16]. These terminals then release several peptides including SP, and CGRP that mediate a local flare response, in concert with histamine from mast cells [3]. Applied to the epineurial plexus, capsaicin induces an intense and prolonged hyperaemic response, a 'peripheral nerve trunk flare' [16].

In this work, we evaluated capsaicin-induced neurogenic inflammation in vasa nervorum of streptozotocin (STZ)-induced diabetic rats. The response to capsaicin was evaluated by making local and serial measurements of endoneurial blood flow (EBF) using hydrogen-sensitive microelectrodes. In addition we made electrophysiological measurements of sural myelinated and unmyelinated conduction to assess the degree of associated neuropathy.

Table 1. Final body weights and blood glucose values 4 months following streptozotocin (diabetic rats) or citrate buffer (control)

	Diabetic rats	Control rats
<i>n</i>	10	8
Weight (g)	266 ± 14 ^a	611 ± 21
Glucose (mmol/l)	23.8 ± 1.6 ^a	6.9 ± 0.3

^a *p* < 0.0001 vs control rats, Values are means ± SEM**Table 2.** Sural nerve conduction studies

	Diabetic rats	Control rats
<i>Myelinated conduction</i>		
<i>n</i>	8	6
CV (m/s)	53.4 ± 1.9 ^a	64.9 ± 3.7
Amplitude (mV)	2.45 ± 0.49	2.93 ± 0.68
<i>Unmyelinated conduction</i>		
<i>n</i>	6	5
CV (m/s)	1.04 ± 0.08 ^b	1.50 ± 0.09
Amplitude (μV)	50.5 ± 15.0	59.3 ± 17.7

^a *p* = 0.006, ^b *p* = 0.002 vs control rats. Results are means ± SEM. CV, Conduction velocity**Table 3.** Endoneurial blood flow, microvascular resistance and mean arterial pressure

	Diabetic rats	Control rats
<i>n</i>	10	8
<i>Endoneurial blood flow</i> (ml · 100 g ⁻¹ · min ⁻¹)		
Pre-capsaicin	19.6 ± 2.1	16.9 ± 2.1 ^a
Post-capsaicin	22.5 ± 2.5	23.6 ± 2.5
<i>Endoneurial microvascular resistance</i> (mm Hg · 100 g · min ⁻¹ · ml ⁻¹)		
Pre-capsaicin	6.67 ± 0.82	7.71 ± 1.12 ^b
Post-capsaicin	5.83 ± 0.53	5.87 ± 0.59
<i>Mean arterial pressure</i> (mm Hg)		
Pre-capsaicin	117 ± 5	116 ± 5 ^c
Post-capsaicin	120 ± 3	129 ± 1

^a *p* = 0.003, ^b *p* = 0.037 pre-vs post-capsaicin, ^c *p* = 0.03 pre-vs post-capsaicin (two-tailed test). Results are means ± SEM

Materials and methods

Male Sprague-Dawley rats of initial weight 250–350 g were given i. p. injections of STZ (65 mg/kg) in citrate buffer at pH 4.5 and accepted as diabetic if blood glucose was greater than or equal to 16.0 mmol/l (measured 5–7 days after STZ). Controls were injected with citrate buffer alone. Glucose measurements were made from the ventral caudal vein using a glucometer (Accu-Chek IIm; Boehringer Mannheim Canada, Dorval, Quebec, Canada) and the oxygen rate method (Beckman Glucose Analyser 2; Beckman Instruments Inc., Palo Alto, Calif., USA). Rats were housed in grouped wire cages with free access to rat chow and water. After 16 weeks of diabetes, measurements of in vitro sural sensory conduction velocity and compound nerve action potential amplitudes were made in myelinated and unmyelinated fibres at 37°C. The electrophysiological techniques have been described in our previous work [14].

Electrophysiological studies and blood flow studies were conducted in rats anaesthetized with sodium pentobarbital (65 mg/kg i. p.). The rats were then prepared for local measurements of endo-

neurial blood flow in the contralateral sciatic nerve using microelectrodes linearly sensitive to hydrogen clearance. Our experimental preparation has previously been described [16]. In brief, anaesthetized rats (as above) underwent tracheostomy and placement of a left carotid intra-arterial catheter, then were paralysed using tubocurarine (1.5 mg/kg intra-arterial) and ventilated. Arterial blood gases were drawn from the catheter every 30–60 min to ensure that values were within an acceptable physiological range (i.e. pO₂ > 90 torr; pCO₂ 35–45 torr; pH 7.35–7.45). Mean arterial blood pressure (MAP) was continuously recorded from the carotid catheter. Supplemental doses of pentobarbital (20 mg/kg) were given approximately every 2 h to maintain a stable level of anaesthesia (as judged by the level and stability of MAP). Local EBF was measured in the mid portion of the sciatic nerve just above its trifurcation. The left sciatic nerve was exposed, covered in mineral oil maintained at 37°C, and a linearly sensitive hydrogen microelectrode inserted through an epineurial window into the endoneurium. Hydrogen was added to the ventilatory gas mixture until endoneurial saturation, then turned off to permit recording of the hydrogen clearance curve. Curves were fitted to mono- or biexponential models to provide EBF measurements using a least squares regression program as previously described [16]. Endoneurial microvascular resistance (EMR) was calculated as MAP/EBF.

In each animal, three clearance curves were recorded. The first curve was routinely discarded, and the second served as a baseline control measurement. As in previous work, the third curve was preceded by bathing the epineurium with 2% capsaicin in 10% ethanol and 10% Tween 80. The carrier does not significantly alter hydrogen clearance [16]. Capsaicin was applied to the epineurium 5 min before the onset of the clearance curve and was left in situ over the epineurium until hydrogen clearance was completed.

Statistical analysis

Data were analysed by determining means and standard errors for electrophysiological results, EBF, MAP and EMR and changes in EBF and EMR following capsaicin. Diabetic and non-diabetic electrophysiological results were compared using an unpaired one-tailed Student's *t*-test. Choice of the one-tailed test was justified by the expected direction of change in these parameters with diabetes [13]. Paired one-tailed Student's *t*-tests were used to compare EBF and EMR measurements before and after capsaicin. In previous work using non-diabetic animals, capsaicin was associated with a rise in EBF and a fall in EMR [16] justifying the choice of a one-tailed test in the present work.

Results

Diabetic rats gained less weight than controls (Table 1), and developed cataracts. Conduction velocities in sural myelinated and unmyelinated fibres were significantly lower in diabetic rats than controls after 16 weeks of diabetes. There was a non-significant trend toward lower myelinated fibre compound nerve action potential amplitudes in diabetics but unmyelinated fibre 'C' potentials were comparable in amplitude between the groups (Table 2). Baseline EBF, EMR and MAP were statistically similar between diabetic and non-diabetic controls. Following the application of epineurial capsaicin, there was a significant rise in EBF (increased by 28%) and a fall in EMR (fell by 24%) over baseline measurements in non-diabetic animals, indicating vasodilation (Table 3). These changes were similar to results previously obtained in non-diabetic rats (EBF increased by 37% and EMR fell by 23% [16]). A mild rise in MAP accompanied these

changes. Although mean EBF rose slightly and mean EMR fell slightly in diabetic animals after capsaicin, changes in individual animals were inconsistent and the difference between pre- and post-capsaicin EBF and EMR were not statistically significant (Table 3).

Discussion

The findings of this study were: 1. diabetic rats developed expected slowing of sural myelinated sensory fibre conduction but also had slowing of unmyelinated fibre conduction; 2. baseline endoneurial blood flow was not altered by diabetes (there was a non-significant trend toward higher flow in the diabetic animals); 3. there was no consistent hyperaemic response to epineurial capsaicin in diabetic rats.

Although mean EBF in diabetic rats increased slightly after capsaicin, a consistent and statistically significant rise did not occur. These findings are not unexpected. Several mechanisms could interrupt capsaicin-induced peptide hyperaemia in diabetes. Neuropathy could reduce the numbers of perivascular nerve terminals or their contents of CGRP (calcitonin gene-related peptide), and SP (substance P) that are released by capsaicin. Our findings indicate that there is involvement of unmyelinated fibres in diabetes: perivascular fibres in the epineurial plexus could indeed be abnormal. Reduction of the content of these peptides in nerve terminals or dystrophic changes in their axons from other tissues of diabetic animals have been noted [17, 18]. Milner et al. [19], however, found increased, rather than decreased SP and CGRP levels in the sheaths of sciatic nerves from rats having diabetes for 8 weeks.

It is possible that perivascular nerve terminals are insensitive to the depolarizing action of capsaicin or that diabetic microvessels become mechanically rigid and lose their capacity to dilate under physiological conditions. In human diabetic sural nerve, microvessels have basement membrane thickening and there is evidence that endoneurial collagen, possibly through glycation, forms more rigid endoneurial structures [20–22]. Other features of diabetic microangiopathy could include non-specific alterations in the smooth muscle contractile apparatus, the availability of calcium, changes in smooth muscle receptor properties or other abnormalities [23]. Indeed, several studies have identified abnormalities in the vasoreactivity and appearance of large and small blood vessels in diabetic tissues indicating the presence of a 'functional microangiopathy' [24–30]. Gamse et al. [31], noted that STZ diabetes in rats abolished both plasma extravasation in rat abdominal skin induced by the direct application of SP and the potentiation of the SP action by CGRP. These findings might suggest that there is an alteration of peptide receptors on mast cells, a change in mast cell numbers, or direct changes of blood vessels in diabetes.

Dysfunction of endothelial mediated vasodilation and contraction could also account for a 'functional microangiopathy'. A common defect at the endothelial level was suggested by Mayhan [27]. SP, CGRP and histamine mediate vasodilation through intact endothelium by releasing

nitric oxide [4–6]. Recent evidence has indicated that nitric oxide is quenched by advanced glycation products and inactivated [32]. Finally, endothelial cell dysfunction in diabetes might also impair the generation of nitric oxide [5].

Lowered baseline EBF using hydrogen clearance has been reported in a model of STZ-induced diabetic neuropathy similar to ours [11, 12]. Although this finding has apparently been confirmed by other groups [33, 34], technical considerations may not render the confirmation valid. Normal 'baseline' EBF has been a consistent feature of our studies in large numbers of rats rendered diabetic by STZ [13, 14] or in BB Wistar rats with genetic diabetes (Zochodne and Ho, unpublished data). Normal nerve EBF has also been reported by Pugliese et al. [15] and morphological appearances of microvessels in experimental diabetes (in contrast to functional changes discussed above) have been unimpressive [35]. It is possible that these discrepancies among investigators may result from other factors: levels of hyperglycaemia in the rats, differences in the genetic strains of the rats used, or variations in the physiological status of the rats. It is also possible that our normal baseline hydrogen clearance EBF measurements could have missed subtle microangiopathy. Plasma skimming of erythrocytes at microvascular bifurcations could result in the clearance of hydrogen by plasma alone [36]. Despite these problems hydrogen clearance provides selective quantitative blood flow information about the endoneurial nutritive circulation and permits serial comparison measurements as in this work. Nonetheless, it would also be important to evaluate the capsaicin flare in genetic diabetes of rats, where the STZ toxin has not been given (although it is unlikely to have influenced vascular responsiveness 4 months after its administration and does not alter nerve conduction without diabetes [14]).

Thus, although there is powerful evidence that microangiopathy does occur in diabetes, an aetiological relationship between changes in nerve perfusion and conduction abnormalities in experimental diabetes remains uncertain. Microangiopathy, however, could play a critical role in the response of nerve to injury.

Our finding that there was loss of the capsaicin 'flare' in diabetic peripheral nerve is consistent with findings in other diabetic tissues that neurogenic inflammation is impaired [8, 9, 31]. Following tissue injury, a Lewis flare is mediated through an 'axon reflex' or 'axon response' accomplished by the perivascular afferent terminal release of SP and CGRP and histamine release by mast cells. In non-diabetic animals, there is evidence that local blood flow is maintained and enhanced following focal nerve injury partly through the action of CGRP [37, 38]. Thus, a 'neurogenic flare' may also be an important component of the peripheral nerve trunk response to injury. Local peptidergic actions may thus have important compensatory functions in injury, such as increasing local tissue content of oxygen, recruiting of macrophages and allowing entry of trophic agents through the normally tight blood-nerve barrier. Loss of the neurogenic injury flare in diabetes could slow regenerative events attempted by endoneurial axons.

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